Scientia Pharmaceutica

ÖSTERREICHISCHE PHARMAZEUTISCHE GESELLSCHAFT

MDPI

Article

Neuropharmacological Activity of the Acetonic Extract of *Malpighia mexicana* A. Juss. and Its Phytochemical Profile

Dante Avilés-Montes [1], David Osvaldo Salinas-Sánchez [2,3,*], César Sotelo-Leyva [4], Alejandro Zamilpa [5], Franceli Itzel Batalla-Martinez [3], Rodolfo Abarca-Vargas [6], Juan Manuel Rivas-González [3], Óscar Dorado [7], Rodolfo Figueroa-Brito [8], Vera L. Petricevich [6], Dulce Lourdes Morales-Ferra [5] and Manasés González-Cortazar [4,*]

1 Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca 62209, Morelos, Mexico; dante.aviles@uaem.mx
2 Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca 62209, Morelos, Mexico
3 Escuela de Estudios Superiores del Jicarero, Universidad Autónoma del Estado de Morelos, Galeana-Tequesquitengo S/N, Col. El Jicarero, Jojutla 62909, Morelos, Mexico; franceli.batalla@uaem.edu.mx (F.I.B.-M.); manuel.rivas@uaem.mx (J.M.R.-G.)
4 Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Av. Lázaro Cárdenas S/N, Ciudad Universitaria Sur, Chilpancingo 39000, Guerrero, Mexico; cesarsotelo@uagro.mx
5 Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, Argentina No. 1, Col. Centro, Xochitepec 62790, Morelos, Mexico; azamilpa_2000@yahoo.com.mx (A.Z.); lou_ferra@hotmail.com (D.L.M.-F.)
6 Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Leñeros, Esquina Iztaccíhuatl S/N, Col. Volcanes, Cuernavaca 62350, Morelos, Mexico; rodolfo.abarca@uaem.mx (R.A.-V.); vera.petricevich@uaem.mx (V.L.P.)
7 Centro de Educación Ambiental e Investigación Sierra de Huautla, Universidad Autónoma del Estado de Morelos, Cuernavaca 62209, Morelos, Mexico; odorado@uaem.mx
8 Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Carretera Yautepec-Jojutla Km 6, AP 24, San Isidro, Yautepec 62731, Morelos, Mexico; rfigueroa@ipn.mx
* Correspondence: davidos@uaem.mx (D.O.S.-S.); gmanases2000@gmail.com (M.G.-C.); Tel.: +52-(777)-329-7019 (D.O.S.-S.); +52-(777)-361-2155 (M.G.-C.)

Abstract: Mental and neurological disorders are conditions that affect thoughts, emotions, behavior, and relationships. *Malpighia mexicana* A. Juss. is a plant used in Mexican traditional medicine for the treatment of such disorders. This work aimed to investigate the antidepressant, anxiolytic, sedative, hypnotic, and anticonvulsant effects of the acetonic extract (MmAE) of *M. mexicana* and its fractions (F3, F4-10, F14) using the forced swimming test (FST), elevated plus maze (EPM), open field test (OFT), pentobarbital-induced sleep test (PBTt), and pentylenetetrazol-induced seizure test (PTZt). MmAE, F3, F4-10, F14, and vehicle were administrated orally 24, 18, and 1 h prior to the evaluations. Imipramine (15 mg/kg, *p.o.*) was administrated 1 h prior to the evaluations as a positive control for the FST, while diazepam (1 mg/kg, *p.o.*) was administrated 1 h prior to the evaluations as a positive control for the EPM, OFT, PBTt, and PTZt. MmAE had an anxiolytic effect; MmAE and F3, F4-10, and F14 showed an antidepressant effect, sedative effect, hypnotic effect, and anticonvulsant effect. Using HPLC, we identified the compounds quercetin 3-*O*-rutinoside (**1**), kaempferol 3-*O*-glucoside (**2**), luteolin 7-*O*-glucoside (**3**), quercetin (**4**), and kaempferol (**5**) in MmAE and compounds (**1**), (**2**), and (**3**) in F14. Using GC-MS, we identified α-tocopherol, phytol, and β-amyrin in F3; β-tocopherol, phytol, β-sitosterol, and β-amyrin in F4-10; and α- tocopherol, phytol, β-sitosterol, and β-amyrin in F4-10. The neuropharmacological effects found in this work may be due to the presence of vitamins, phytosterols, terpenes, and flavonoids. This research requires further study to clarify the mechanisms of action of the identified compounds.

Keywords: antidepressant; anxiolytic; sedative; hypnotic; anticonvulsant; *Malpighia mexicana*

Citation: Avilés-Montes, D.; Salinas-Sánchez, D.O.; Sotelo-Leyva, C.; Zamilpa, A.; Batalla-Martinez, F.I.; Abarca-Vargas, R.; Rivas-González, J.M.; Dorado, Ó.; Figueroa-Brito, R.; Petricevich, V.L.; et al. Neuropharmacological Activity of the Acetonic Extract of *Malpighia mexicana* A. Juss. and Its Phytochemical Profile. *Sci. Pharm.* **2023**, *91*, 47. https://doi.org/10.3390/scipharm91040047

Academic Editors: Marta Menegazzi and Sonia Piacente

Received: 23 August 2023
Revised: 25 September 2023
Accepted: 28 September 2023
Published: 10 October 2023

1. Introduction

Malpighia mexicana A. Juss. (Malpighiaceae), is distributed in Chiapas, Durango, Mexico State, Guerrero, Jalisco, Michoacán, Morelos, Oaxaca, Puebla, and Yucatán [1,2]. Its common name is guachocote, and it has edible fruits that have a high vitamin C content [2]. It is used in traditional medicine against stomach ailments, diabetes, and scurvy, and the people of the communities within the Sierra de Huautla Biosphere Reserve in Morelos (REBIOSH), Mexico, use it for the treatment of nervous disorders [3]. However, its use is empirical, and to date, there are no scientific publications that report its effectiveness against the ailments it is used to treat. *M. mexicana* A. Juss. grows wild, but rural inhabitants also cultivate it in home gardens because of its multiple economic, food, timber, ornamental, and medicinal benefits [1]. Several studies of plant species belonging to different genera of the Malpighiaceae family have reported uses in traditional medicine for various nervous disorders and found sedative, antidepressant, anxiolytic, anticonvulsant, nootropic, and learning-enhancement effects [4,5].

Plant species are a valuable resource for large pharmaceutical companies in the development of new drugs. They are used as a direct source of therapeutic agents (both as phytomedicines and pure drugs), a source of raw material for the development of complex semi-synthetic drugs, prototypes for the design of lead molecules, and/or taxonomic markers for the discovery of new drugs [6].

Depression and anxiety disorders are the most frequent mental illnesses worldwide [7]. Anxiety affects more than 264 million people, with up to 33.7% of the world's population estimated to experience an anxiety disorder during their lifetime [8,9]. Both depressive disorders and anxiety are among the top ten causes of disease burden worldwide, with depression being the second leading cause and anxiety being the fifth leading cause of lost years due to disability [10]. Furthermore, many people have both conditions [11]. Epilepsy is one of the most common neurological disorders, with more than seventy million people suffering worldwide. Its incidence has a bimodal distribution with respect to age, with the highest risk in children and older adults [12]. For most people with epilepsy, anticonvulsant medications are the main treatment modality to stop seizures as soon as possible without causing side effects [12]. There are different pharmacological treatments for these disorders; however, some have limited efficacy, and some patients are refractory to multiple treatments at typical doses [13]. Unfortunately, many patients present adverse effects to pharmacotherapy; these include sedation, fatigue, cardiovascular alterations, erectile dysfunction, anorgasmia and delayed ejaculation with tricyclic antidepressants; insomnia, increased anxiety, irritability, and decreased libido with selective serotonin reuptake inhibitors; and sedation, mild memory impairment, decreased alertness, and myorelaxation with benzodiazepines [14–16].

The objective of the present research was to determine the antidepressant, anxiolytic, hypnotic, and anticonvulsant activity of the innocuous acetonic extract and two innocuous fractions isolated from the leaves of *M. mexicana* A. Juss. in CD−1 mice, using a pharmacological battery that consisted of the elevated plus maze (EPM), forced swimming test (FST), open field test (OFT), pentobarbital-induced sleep test (PBTt), and pentylenetetrazol-induced seizure test (PTZt). The MmAE extract and F14 were analyzed by HPLC, and F3 and F4-10 were analyzed by GC-MS to identify their constituents.

2. Materials and Methods

2.1. Plant Collection and Identification

Leaves of *M. mexicana* A. Juss. were collected in the south of the state of Morelos in the community of Chiconcuac, municipality Xochitepec, in September 2021 (latitude N 18°47′3.9766″ longitude W 99°11′42.193″ Altitude: 1171 m.a.s.l.). The identification of the plant was authenticated by M.C. Gabriel Flores, an expert in the field of plant taxonomy. One specimen was deposited as a reference in the herbarium (HUMO) of the Biodiversity and Conservation Research Center of the Autonomous University of the State of Morelos, with accession number 35164.

2.2. Preparation of MmAE Extract

The dried and ground material (150 g) was extracted with acetone by the maceration method for 3 days/3 times. The solvent was completely removed by distillation under a reduced-pressure distillation system with the aid of a Buchi R-215 rotary evaporator (EQUIPAR, CDMX, Mexico) and subsequent high vacuum drying. The MmAE extract was then stored at 4 °C until use in the pharmacological models. The MmAE extract was dissolved with a 1% NaCl saline solution (vehicle) and was sonicated with ultrasonic movements in an Ultrasonic Cleaner with Mechanical Timer (Cole-Parmer model 08895-21), (SHARPER TEK, Pontiac, MI, USA).

The selection of the initial doses of MmAE was taken from a previous work carried out with *Heteropterys brachiata* (L.), a plant of the same botanical family as *Malpighia mexicana* (Malpighiaceae). The authors performed neuropharmacological evaluations of a methanolic extract and evaluated doses 500, 750, 1000, and 1500 mg/kg, *p.o.*, and found antidepressant, anxiolytic, and anticonvulsant effects [17]. It was decided to perform an acute toxicity evaluation of MmAE with doses of 100, 200, 400, and 600 mg/kg, *p.o.*, (Figure S16). Based on the results obtained in each pharmacological model, the best dose was chosen to evaluate fractions F3, F4-10, and F14.

2.3. MmAE Fractionation

The MmAE (8 g) was fractionated on a chromatographic column previously packed with 40 g of silica gel 60. An *n*-hexane-acetone gradient system was used as the mobile phase, starting with 100% of the lower polarity solvent, subsequently eluting with *n*-hexane-acetone gradients of 95:5%–90:10%–85:15% until reaching 50:50% *n*-hexane–acetone, 70:30% acetone–*n*-hexane, and 100% acetone and finally washing with 100% methanol, resulting in 14 fractions of 100 mL each. The solvent was completely removed by distillation under a reduced-pressure distillation system with the aid of a Buchi R-215 rotary evaporator and subsequent high vacuum drying. The fractions were subsequently analyzed in thin-layer chromatography and were grouped based on similar patterns in their chemical composition, resulting in three sub-fractions, F3, F4-10, and F14.

2.4. HPLC and UPLC Analysis

HPLC analysis of the MmAE extract was used to detect the constituents present. HPLC was carried out on a Waters 2695 liquid chromatograph (Waters, Milford, MA, USA) equipped with a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). The separation was carried out using an RP C-18 Superspher (Merk) column (120 × 4 mm; 5 µm) (Merck, Darmstadt, Germany). For the mobile phase, solvent A was HPLC-quality water plus 0.5% and solvent B was acetonitrile. We used the following solvent ratios: A:B = 100:0 (0–1 min); 95:05 (2–3 min); 70:30 (4–20 min); 50:50 (21–22 min); 20:80 (24–25 min); 0:100 (26–27 min), and 100:0 (28–30 min). The injection sample volume was 10 µL with a flow rate of 0.9 mL/min. The detection wavelength was recorded as 200–400 nm. The commercial standards used were quercetin 3-*O*-rutinoside (R5143) (**1**), kaempferol 3-*O*-glucoside (PHL89237) (**2**), luteolin 7-*O*-glucoside (1370837) (**3**), quercetin (Q4951) (**4**), and kaempferol (K0133) (**5**) from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The sample was prepared at a concentration of 1 mg/mL using HPLC-grade methanol with 0.05% formic acid, and 5 µL was injected.

For UPLC, the equipment used was a UPLC ACQUITY instrument (Waters Corporation, Milford, MA 01757, USA). The chromatographic system consisted of a quaternary pump (972A), a column oven with an autosampler (8126), a degasser, and a photodiode array detector (436 A). The column used was an ACQUITY UPLC®BEHC18 column (1.7 mn × 50 mm × 2.1 mm). The column was operated at a flow rate of 0.3 mL/min and the system was thermostatted at 30 °C. The mobile phase that was used for the separation was a gradient system using two phases: phase A (UPLC water + 0.05% trifluoroacetic acid) and phase B (acetonitrile). The elution system was as follows: 0.0–1.0 min, 100% A; 2.0–14.0 min, 70% A; 15.0 min, 30% A, 16.0 min, 0% A, 17.0–20.0 min, 100% A.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of F3 and F4-10 was analyzed by gas chromatography-mass spectrometry (GC-MS) following the method described by Gallegos-García, et al. [18]. The equipment consisted of an Agilent 6890 plus gas chromatograph coupled to a simple quadrupole mass spectrometry detector, model 5972N (Agilent Technology, Santa Clara, CA, USA). The volatiles were identified by comparing their mass spectra to those of the National Institute of Standards and Technology (NIST) 1.7 Library and data from the literature [19].

2.6. Drugs and Chemicals

Diazepam (DZP), pentylenetetrazole (PTZ), and imipramine (IMI) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium pentobarbital (PBT) was purchased from Pfizer Inc. (New York, NY, USA).

2.7. Animals

Experiments with rodents were carried out in accordance with the Mexican Official Standard (NOM-062-ZOO-1999) "Technical specifications for the production, care, and use of laboratory animals". The experimental protocol was approved by the institutional research and ethics committee (R-2023-1702-008). For each pharmacological experiment, groups of eight male CD−1 albino mice weighing 20–30 g obtained from the biotherium of the Faculty of Medicine of the Autonomous University of the State of Morelos were used. They were housed communally in Plexiglas boxes under laboratory conditions (23 ± 1 °C, 12-h light–dark cycle, with free access to water and standard rodent chow). All animals were acclimatized for 2 weeks prior to experimentation.

2.8. Neuropharmacological Assays

2.8.1. Forced Swimming Test (FST)

Among all animal models, the FST is one of the most widely used tools in screening for antidepressants [20,21]. The apparatus used consisted of a transparent plastic cylinder (17 cm high × 14 cm diameter) filled with water (25 ± 1 °C) to a depth of 15 cm. Mice received their respective treatments 24, 18, and 1 h before the trial. The treatments of MmAE (100, 200, 400, 600 mg/kg, *p.o.*, n = 8), F3 (100, 400 mg/kg, *p.o.*, n = 8), F4-10 (100, 400 mg/kg, *p.o.*, n = 8), F14 (100, 400 mg/kg, *p.o.*, n = 8), s.s. 0.1% (vehicle, *p.o.*, control group, n = 8), and IMI (15 mg/kg, *p.o.*, positive control, n = 8) were always administered 1 h prior to experimentation. All evaluation sessions were recorded with a video camera, and the immobility time(s) during the 5 min test was quantified from the recordings.

2.8.2. Elevated Plus Maze Test (EPM)

The EPM is a widely accepted international model in the search for substances with anxiolytic activity [22–24]. The device was constructed of Plexiglas and consisted of a central platform (5 × 5 cm) with two open arms (30 × 15 cm) and 15-cm-high walls. The maze had a floor elevation of 40 cm. Mice received their respective treatments 24, 18, and 1 h before the trial: MmAE (100, 200, 400, 600 mg/kg, *p.o.*, n = 8), F3 (400 mg/kg, *p.o.*, n = 8), F4-10 (400 mg/kg, *p.o.*, n = 8), F14 (400 mg/kg, *p.o.*, n = 8), s.s. 0.1% (vehicle, *p.o.*, control group, n = 8); DZP treatment (1 mg/kg, *p.o.*, positive control, n = 8) was administered 1 h prior to experimentation. Each animal was placed in the center of the device facing one of the open arms. All evaluation sessions were recorded with a video camera. The test lasted 5 min, and the percentage of number of entries and the percentage of time spent in the open and closed arms were quantified from the recordings.

2.8.3. Open Field Test (OFT)

This test is widely used in the evaluation of motor activity in rodents subjected to different drugs for evidence of a sedative or stimulant effect [25–27]. The device used for this test is a box made of Plexiglas that has a black floor and transparent walls (30 × 30 × 15 cm).

The bottom is divided into nine squares of equal size. The mice received their respective treatments 24, 18, and 1 h before the trial: MmAE (100, 200, 400, 600 mg/kg, *p.o.*, $n = 8$), F3 (400 mg/kg, *p.o.*, $n = 8$), F4-10 (400 mg/kg, *p.o.*, $n = 8$), F14 (400 mg/kg, *p.o.*, $n = 8$), s.s. 0.1% (vehicle, *p.o.*, control group, $n = 8$); DZP (1 mg/kg, *p.o.*, $n = 8$) was administered 1 h before experimentation. All sessions were recorded with a video camera. The total squares crossed by all four paws (crossings) and the number of rearings during 5 min were quantified from the recordings.

2.8.4. Pentobarbital-Induced Sleep Test (PBTt)

This test is used in the search for drugs with the ability to potentiate the hypnotic state of sodium pentobarbital (PBT) [28,29]. The mice received their respective treatments 24, 18, and 1 h before the trial: MmAE (100, 200, 400, 600 mg/kg, *p.o.*, $n = 8$), F3 (400 mg/kg, *p.o.*, $n = 8$), F4-10 (400 mg/kg, *p.o.*, $n = 8$), F14 (400 mg/kg, *p.o.*, $n = 8$), s.s. 0.1% (vehicle, *p.o.*, control group, $n = 8$); DZP (1 mg/kg, *p.o.*, positive control, $n = 8$) was administered 1 h before experimentation. All treatments received PBT (30 mg/kg, *i.p.*), and the mice were individually placed in a clear acrylic box for evaluation for 30 min. Sleep induction time (time elapsed between injection and loss of the righting reflex) and sleep time (time interval between loss and recovery of the righting reflex) were recorded in seconds and minutes, respectively.

2.8.5. Pentylenetetrazol-Induced Seizure Test (PTZt)

This assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that most anxiolytic agents are also capable of preventing or antagonizing PTZ-induced seizures [30,31]. The mice received their respective treatments 24, 18, and 1 h before the trial: MmAE (50, 100, 150, 200, 250 mg/kg, *p.o.*, $n = 8$), F3 (400 mg/kg, *p.o.*, $n = 8$), F4-10 (400 mg/kg, *p.o.*, $n = 8$), F14 (400 mg/kg, *p.o.*, $n = 8$), s.s. 0.1% (vehicle, *p.o.*, control group, $n = 8$); DZP (1 mg/kg, *p.o.*, positive control, $n = 8$) was administered 1 h prior to experimentation. All treatments received PTZ (100 mg/kg, *i.p.*), and the mice were individually placed in a clear acrylic box and observed for seizure onset for a period of 30 min. Latency (time elapsed between PTZ injection and first seizure); number of clonic seizures, tonic seizures, and total seizures; and percentage of mortality protection in mice were recorded.

2.9. Statistical Analysis

All data are represented as the mean ± standard deviation (S.D.). Data were analyzed by one-way analysis of variance (one-way ANOVA) followed by Tukey's test for comparison against control. Values of $p \leq 0.05$ were considered statistically significant. All analyses were performed with Jamovi Stats 2.3.18 (Sydney, Australia).

3. Results

A yield of 3.7% was obtained from the acetonic extract of *Malpighia mexicana* A. Juss. leaves (MmAE) with respect to the dry material.

3.1. HPLC Analysis

3.1.1. HPLC of MmAE

Figure 1 shows the HPLC profile of MmAE recorded at λ = 330 nm, which detected five flavonoid-type peaks that corresponded to quercetin 3-*O*-rutinoside (**1**) with a time of 8.89 min (λ_{max} = 192, 265, and 354 nm), kaempferol 3-*O*-glucoside (**2**) with a time of 9.09 min (λ_{max} = 194, 263 and 351 nm), to luteolin 7-*O*-glucoside (**3**) in 9.46 min (λ_{max} = 192, 265 and 344 nm), quercetin (**4**) in 11.71 min (λ_{max} = 194, 255 and 367 nm), and kaempferol (**5**) in 14.70 min (λ_{max} = 192, 267 and 364 nm). These compounds were identified by direct comparison with commercial standards (Figure 1).

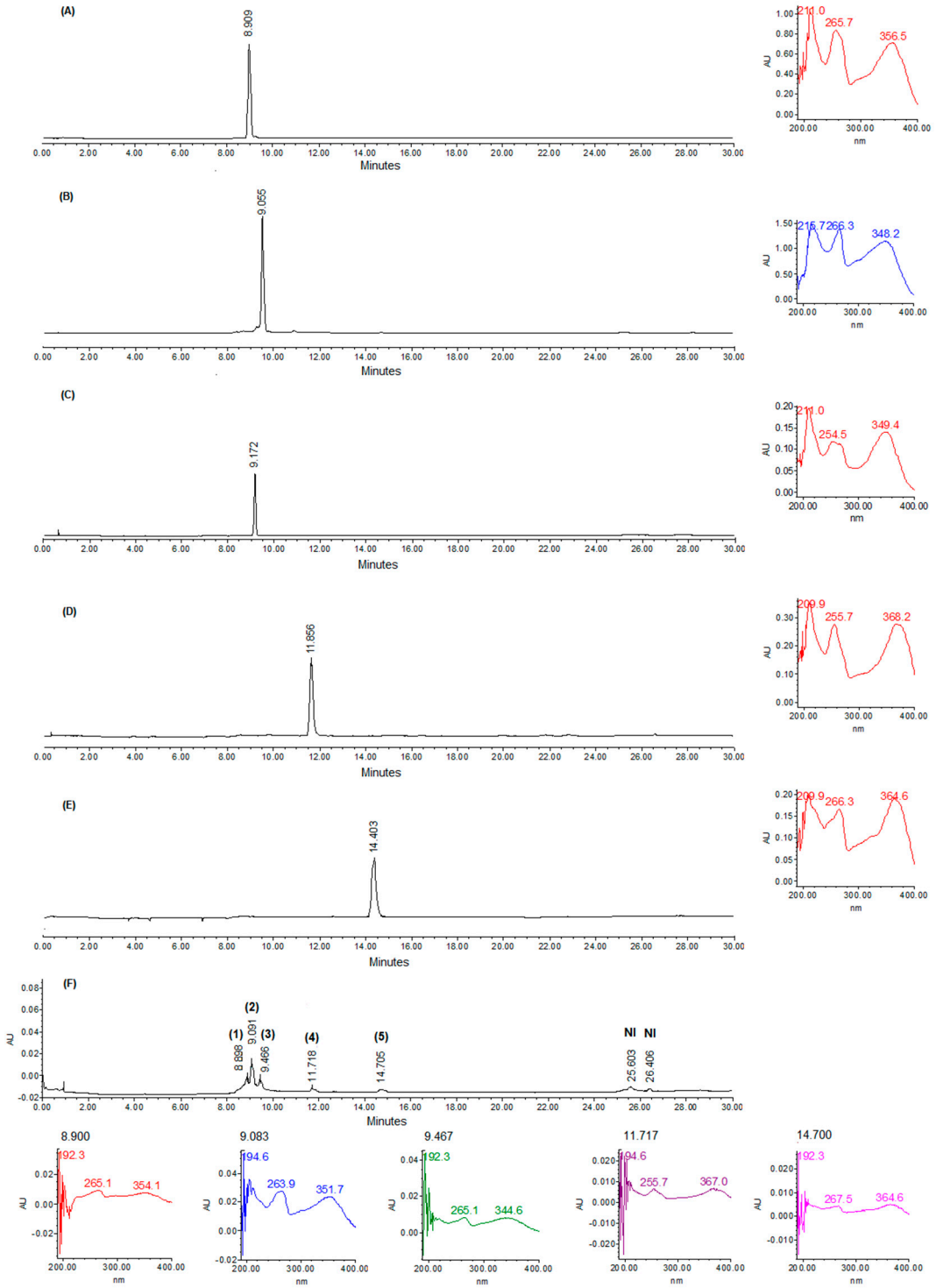


Figure 1. HPLC chromatograms: commercial standards (**A**) quercetin 3-*O*-rutinoside, (**B**) kaempferol 3-*O*-glucoside, (**C**) luteolin 7-*O*-glucoside, (**D**) quercetin, (**E**) kaempferol, and (**F**) acetone extract of *Malpighia mexicana* A. Juss. leaves (MmAE) observed at λ = 330 nm. (**1**) Quercetin 3-*O*-rutinoside, (**2**) kaempferol 3-*O*-glucoside, (**3**) luteolin 7-*O*-glucoside, (**4**) quercetin, (**5**) kaempferol, NI = not identified.

3.1.2. UPLC-MS Analysis

UPLC-MS analysis of MmAE identified the following compounds: kaempferol 3-*O*-glucoside (**2**), lutolin 7-*O*-glucoside (**3**), and kaempferol 3-*O*-rutinoside (Figure 2).

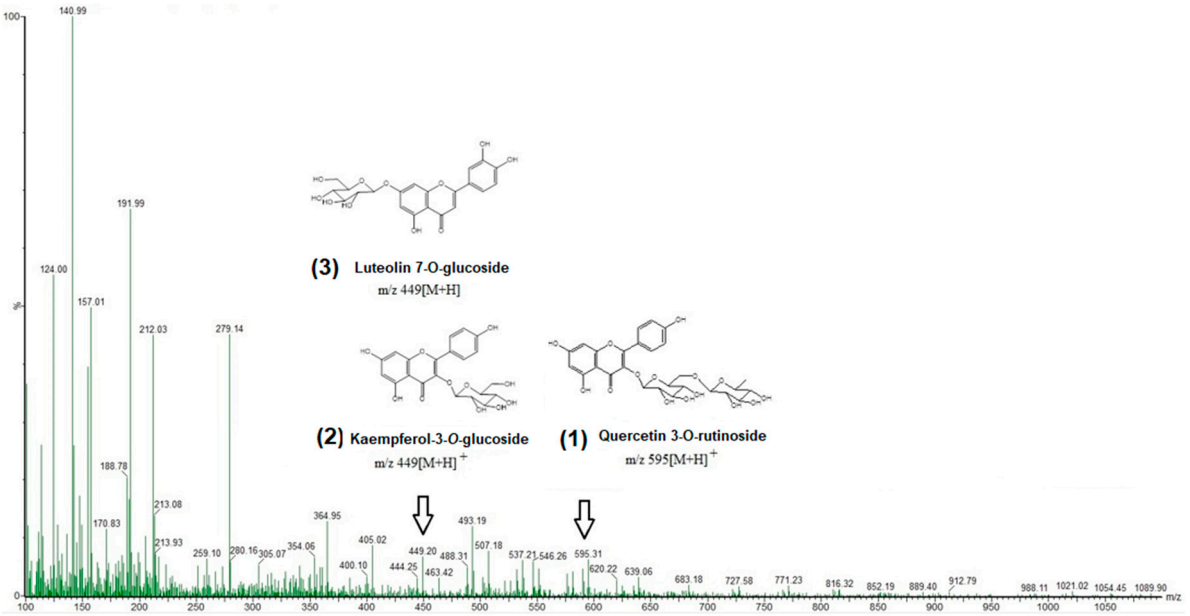


Figure 2. UPLC-MS chromatogram of MmAE. The compounds identified were quercetin 3-*O*-rutinoside (**1**), kaempferol 3-*O*-glucoside (**2**), and luteolin 7-*O*-glucoside (**3**).

3.1.3. HPLC of Fraction 14

The HPLC chromatogram of F14 recorded at λ = 330 nm is shown in Figure 3. The chromatographic analysis identified the same flavonoids as in the whole extract (Figure 1): quercetin 3-*O*-rutinoside (**1**) (Rt = 8.907 min, λ_{max} = 254, 355.3 nm), kaempferol 3-*O*-glucoside (**2**) (Rt = 9.102 min, λ_{max} = 259, 352.9 nm) and luteolin 7-*O*-glucoside (**3**) (Rt = 9.469 min, λ_{max} = 265.1, 347 nm). The UPLC analysis allowed a better separation, identifying five peaks corresponding to (**1**) (Rt = 2.99), (**2**) (Rt = 3.09), (**3**) (Rt = 3.14), (**5**) (Rt = 3.24), and an unidentified compound (Rt = 3.19) (Figure S1).

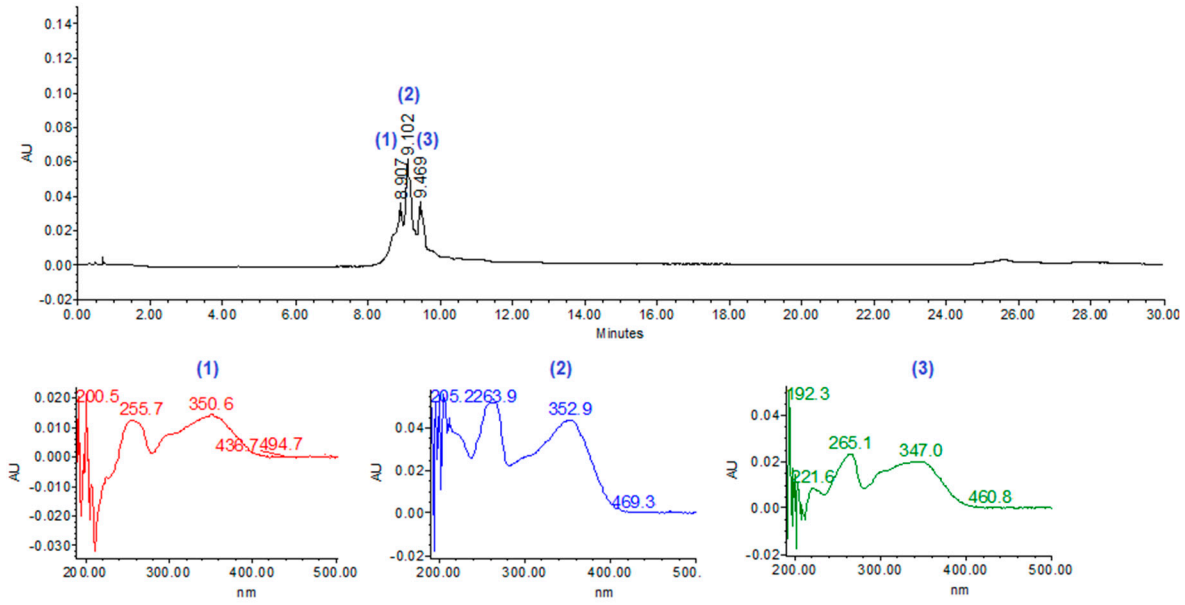


Figure 3. HPLC chromatograms corresponding to the F14 of *Malpighia mexicana* A. Juss. leaves observed at λ = 330 nm. UV spectrum: quercetin 3-*O*-rutinoside (**1**) (Rt = 8.90 min), kaempferol 3-*O*-glucoside (**2**) (Rt = 9.10 min), and luteolin 7-*O*-glucoside (**3**) (Rt = 9.46 min).

3.2. GC-MS of F3 and F4-10

Total ion current (TIC) chromatograms of F3 and F4-10 are shown in Figures 4 and 5. The GC-MS chromatograms (TIC) of F3 allowed the identification of at least 12 compounds, which are listed in Table 1, arranged according to their elution order. Compounds detected for F3 were α-tocopherol (relative abundance: 26.51%), phytol (13.96%), and β-amyrin (12.31%). For the F4-10, GC-MS chromatograms (TIC) allowed the identification of seven compounds (Table 2), which included α-tocopherol (27.241%), phytol (10.754%), and β-sitosterol (9.604%). It is worth noting that this is the first report of a GC-MS analysis of any extract or fraction from *Malpighia mexicana* A. Juss.

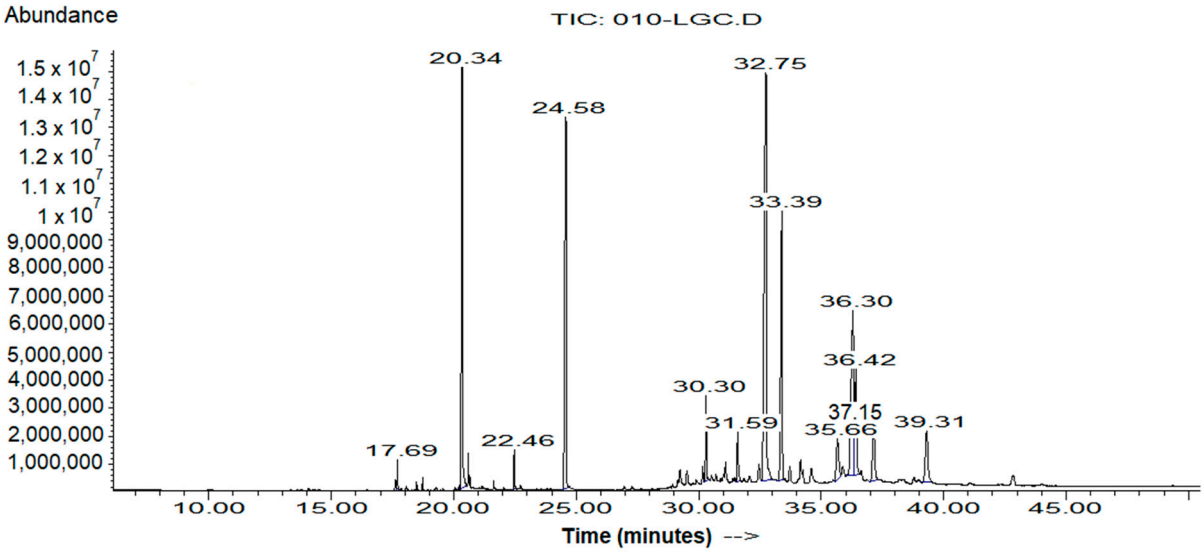


Figure 4. Typical gas chromatography/mass spectrometry (GC/MS) total ion current (TIC) chromatograms of F3 of *Malpighia mexicana* A. Juss.

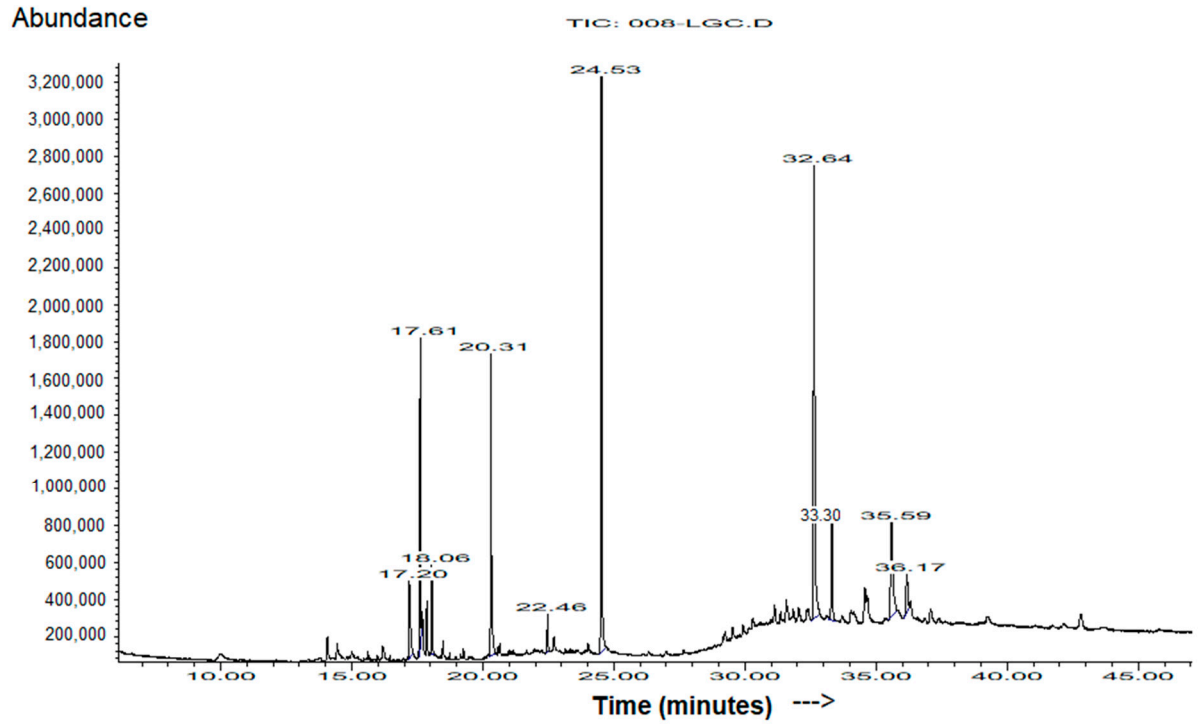


Figure 5. Typical gas chromatography/mass spectrometry (GC/MS) total ion current (TIC) chromatograms of the Fraction 4-10 of *Malpighia mexicana* A. Juss.

Table 1. Compounds identified in F3 of *Malpighia mexicana* A. Juss.

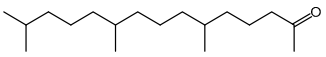
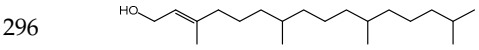
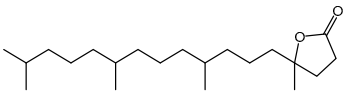
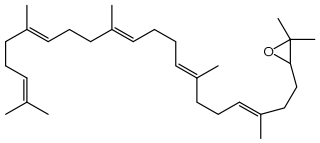
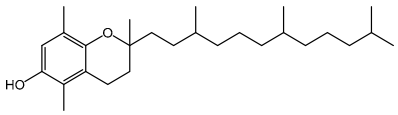
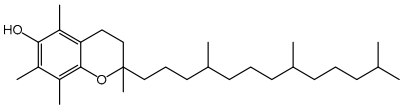
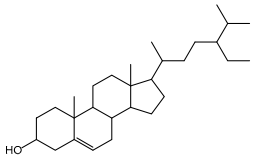
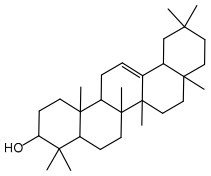
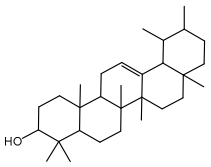
Peak	Compound	Retention Time (min)	Relative Area (%)	Correlation (%)	Mass (g/mol)	Chemical Structure
1	6,10,14-trimethylpentadecan-2-one	17.692	0.5	2.0	268	
2	phytol	20.346	13.9	52.6	296	
3	4,8,12,16-tetramethylheptadecan-4-olide	22.467	0.7	3.0	324	
4	squalene oxide	30.304	2.2	8.4	426	
5	β-tocopherol	31.591	1.6	6.2	416	
6	α-tocopherol	32.747	26.5	100	430	
7	β-sitosterol	35.664	2.7	10.3	414	
8	β-amyrin	36.301	12.3	46.6	426	
9	α-amyrin	37.148	3.6	13.7	426	

Table 2. Compounds identified in the F4-10 of *Malpighia mexicana* A. Juss.

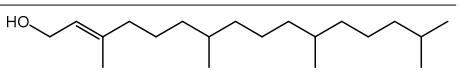
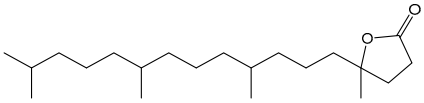
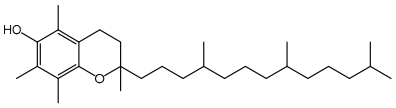
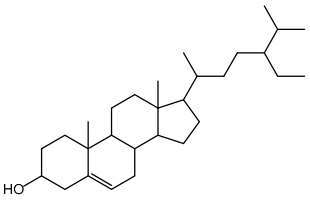
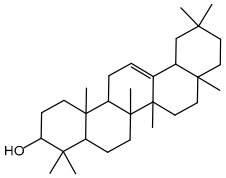
Peak	Compound	Retention Time (min)	Relative Area (%)	Correlation (%)	Mass (g/mol)	Chemical Structure
1	phytol	20.313	10.7	39.4	296	
2	4,8,12,16-tetramethylheptadecan-4-olide	22.467	1.2	4.7	324	

Table 2. *Cont.*

Peak	Compound	Retention Time (min)	Relative Area (%)	Correlation (%)	Mass (g/mol)	Chemical Structure
3	α-tocopherol	32.635	27.2	100	430	
4	β-sitosterol	35.591	9.6	35.2	414	
5	β-amyrin	36.169	2.9	10.4	426	

3.3. Pharmacological Assays

3.3.1. FST

Mice treated with MmAE 100 mg/kg differed in their immobility time compared to the control group (Veh) ($p < 0.05$), and the 200, 400, and 600 mg/kg doses differed strongly from the control group (Veh) ($p < 0.001$), indicating that these treatments had an antidepressant effect during the FST. This result is similar to that yielded by IMI (15 mg/kg) (Figure 6). F3 (100 mg/kg) markedly and significantly reduced the immobility time of mice compared to Veh ($p < 0.001$); F3 (400 mg/kg) also decreased the immobility time ($p < 0.05$), but the greatest effect was obtained with the smallest dose. F4-10 reduced immobility time in a dose-dependent manner (100 mg/kg, $p < 0.05$; 400 mg/kg, $p < 0.001$), and the 400 mg/kg dose reduced immobility time even more than IMI. F14 (100, 400 mg/kg) significantly decreased immobility time compared to Veh ($p < 0.001$); this effect was better than that of IMI. These results indicated that the fractions had an antidepressant effect (Figure 7).

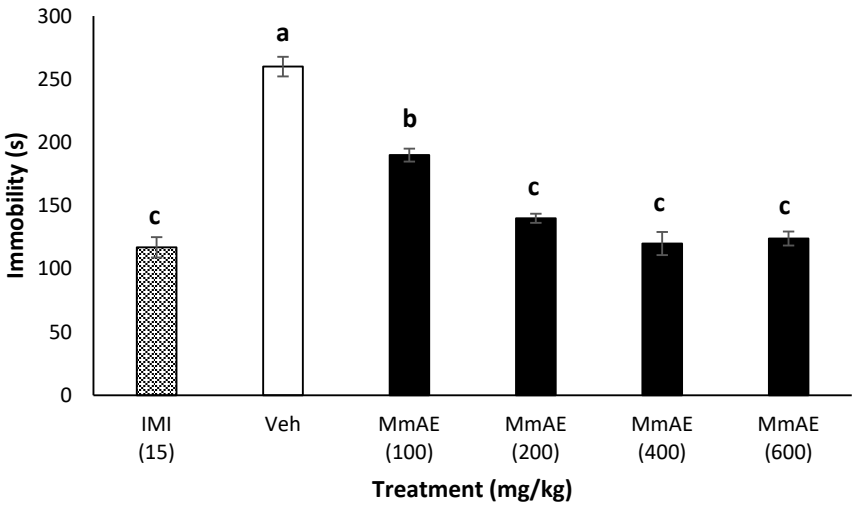


Figure 6. Effect of oral administration of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the immobility time of CD−1 mice exposed to the FST. a = $p > 0.05$; b = $p < 0.05$; c = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. IMI, imipramine hydrochloride; Veh, vehicle (100 μL/10 g); MmAE acetonic extract of *M. mexicana* A. Juss.

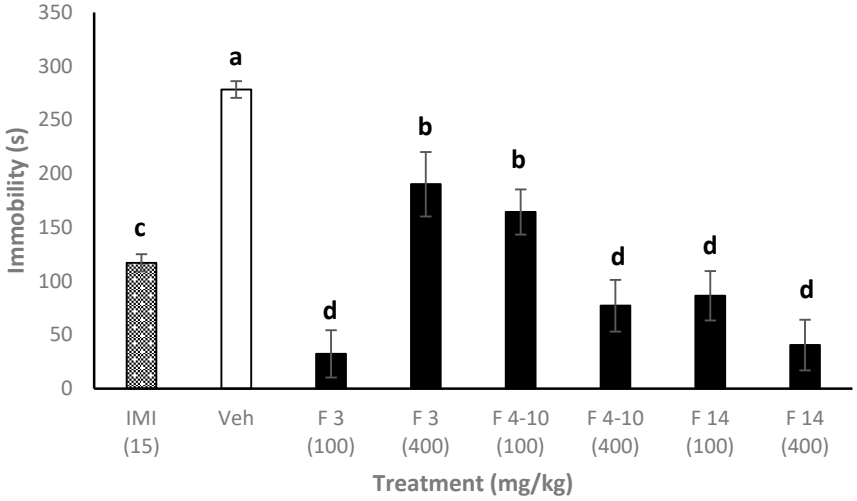


Figure 7. Effect of oral administration of three fractions of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the immobility time of CD−1 mice exposed to the FST. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.05$; c, d = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. IMI, imipramine hydrochloride; Veh, vehicle (100 µL/10 g).

3.3.2. EPM

The anxiolytic DZP induced a significant increase in the percentage of the number of entries into the open arms (EOA) ($p < 0.001$) and the percentage of time spent in open arms (TOA) ($p < 0.001$) in the EPM relative to control mice. MmAE (100 mg/kg) only significantly increased %EOA compared to Veh, MmAE (200 mg/kg) significantly increased %EOA and %TOA ($p < 0.05$), and MmAE (400 mg/kg) presented the best result, significantly increasing %EOA and %TOA compared to Veh ($p < 0.001$). These results are similar to those obtained with DZP. MmAE increased both parameters %EOA ($p < 0.05$) and %TOA ($p < 0.001$). These results indicate that MmAE extract presented a dose-dependent anxiolytic effect (Figure 8). The three chemically simpler fractions of F3 (400 mg/kg), F4-10 (400 mg/kg), and F14 (400 mg/kg) did not affect the EOA or TOA parameters with respect to the control group (Veh) (Figure 9).

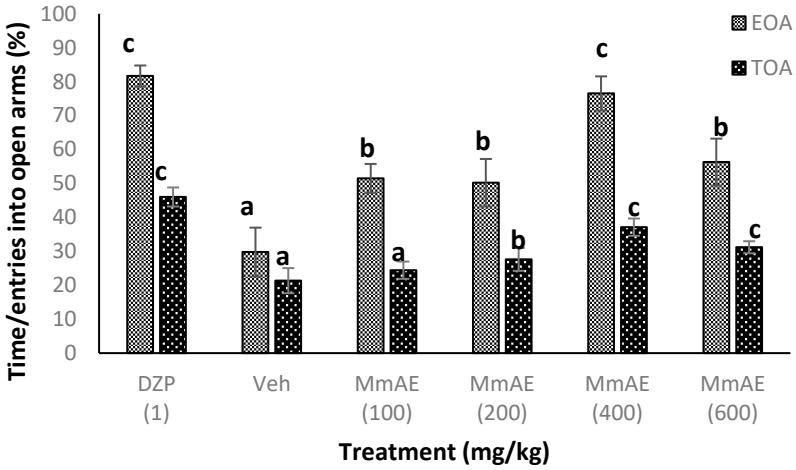


Figure 8. Effect of oral administration of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the percentage of time spent in open arms (TOA) and percentage of entries into open arms (EOA) by CD−1 mice exposed to the EPM. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.05$; c = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. DZP, diazepam; Veh, vehicle (100 µL/10 g); MmAE, acetonic extract of *M. mexicana* A. Juss.

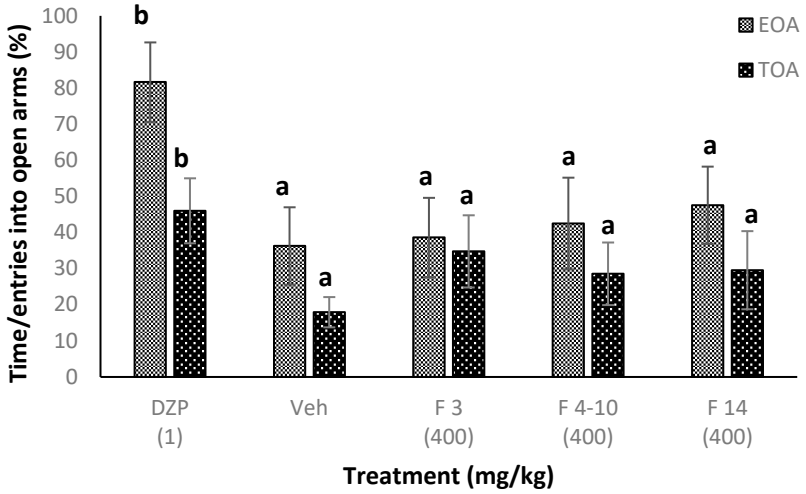


Figure 9. Effect of oral administration of three fractions of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the percentage of time spent in open arms (TOA) and percentage of entries into open arms (EOA) by CD−1 mice exposed to the EPM. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with n= 8. DZP, diazepam; Veh, vehicle (100 µL/10 g).

3.3.3. OFT

DZP (1 mg/kg) did not change the number of total crossings compared to the control group (Veh) ($p > 0.05$), nor did it induce differences in the number of rearings compared to Veh ($p > 0.05$). Mice treated with 100, 200, 400, and 600 mg/kg of MmAE exhibited a decreased in the number of total crossings ($p < 0.001$) but no difference in the number of rearings when compared to the control (Veh) mice (Figure 10). Similarly, when the chemically simpler fractions F3 (400 mg/kg), F4-10 (400 mg/kg), and F14 (400 mg/kg) were evaluated, they exhibited a decrease in the number of total crossings ($p < 0.001$) but no difference in the number of rearings when compared to the control (Veh) (Figure 11).

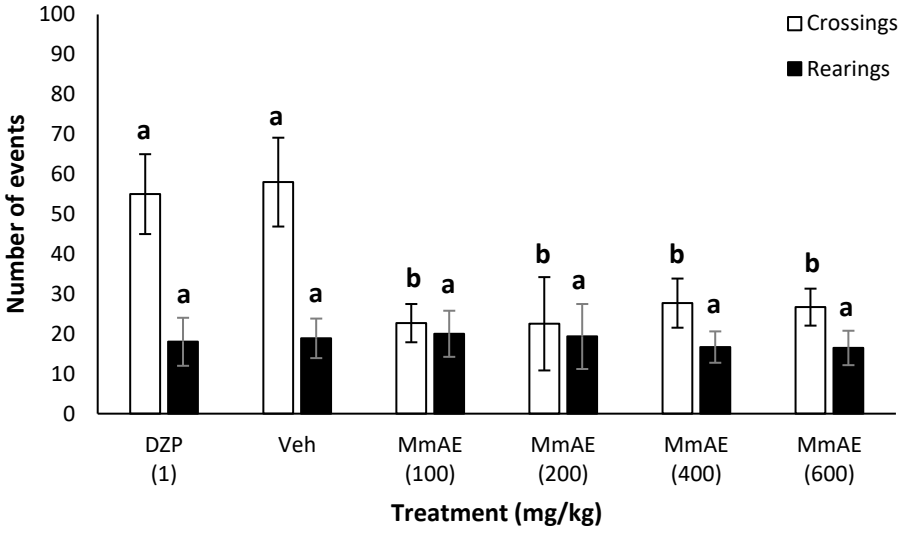


Figure 10. Effect of oral administration of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the total number of crossings and rearings of CD−1 mice exposed to the OFT. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with n = 8. DZP, diazepam; Veh, vehicle (100 µL/10 g); MmAE, acetonic extract of *M. mexicana* A. Juss.

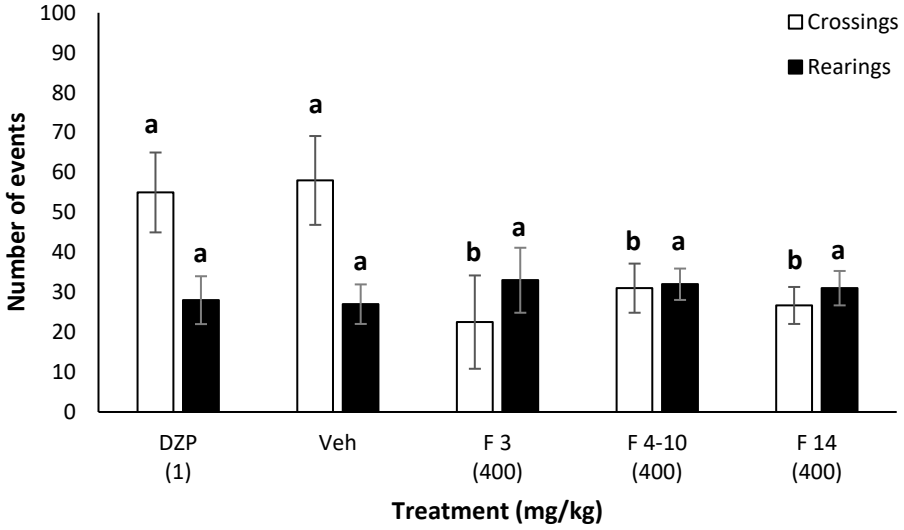


Figure 11. Effect of oral administration of the fractions F3, F4-10, and F14 on the total number of total crossings and rearings of CD−1 mice exposed to OFT. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. DZP, diazepam; Veh, vehicle (100 µL/10 g).

3.3.4. PBTt

The control group (Veh) did not lose the righting reflex with pentobarbital administration (30 mg/kg, *i.p.*), and thus did not exhibit latency (0.0 ± 0.0) or sleep time duration (0.0 ± 0.0). The DZP group (1 mg/kg) potentiated the hypnotic effect of pentobarbital and presented a latency time of 189.37 ± 53.53 s ($p < 0.001$) and a sleep duration of 1610.625 ± 53.53 s ($p < 0.001$). MmAE (100 mg/kg) presented a latency time of 489.80 ± 19.37 s ($p < 0.001$) and a sleep duration of 1055.20 ± 32.94 s ($p < 0.001$). MmAE (200 mg/kg) presented a latency time of 398.40 ± 23.98 s ($p < 0.001$) and a sleep duration of 1208.80 ± 33.89 s ($p < 0.001$). MmAE (400 mg/kg) presented a latency time of 399.20 ± 85.94 s ($p < 0.001$) and a sleep duration of 1169.60 ± 44.38 s ($p < 0.001$). MmAE (600 mg/kg) presented a latency time of 494.20 ± 64.13 s ($p < 0.001$) and a sleep duration of 1138.00 ± 55.95 s ($p < 0.001$) (Figure 12). The chemically simpler fractions F3 (400 mg/kg), F4-10 (400 mg/kg), and F14 (400 mg/kg) were evaluated and induced the loss of the righting reflex, significantly affecting the latency and duration of pentobarbital sodium-induced sleep compared to the control (Veh) ($p < 0.001$). F3 (400 mg/kg) presented a latency time of 173.83 ± 25.63 s ($p < 0.001$) and a sleep duration of 1626.16 ± 25.63 s ($p < 0.001$). F4-10 (400 mg/kg) presented a latency time of 206.50 ± 20.00 s ($p < 0.001$) and a sleep duration of 1593.50 ± 50.78 s ($p < 0.001$). F14 (400 mg/kg) presented a latency time of 214.50 ± 26.37 s ($p < 0.001$) and a sleep duration of 1585.50 ± 26.37 s ($p < 0.001$) (Figure 13).

3.3.5. PTZt

Doses of 50 and 100 mg/kg MmAE induced a significant reduction in the number of clonic seizures ($p < 0.001$) compared with Veh; this result is similar to the effect produced by DZP (1 mg/kg). MmAE (150, 200, and 250 mg/kg) also significantly decreased the number of clonic seizures ($p < 0.05$) (Figure 14). DZP (1 mg/kg) increased seizure latency time (131.75 ± 7.68 s) compared with Veh ($p < 0.05$). MmAE (50, 100, 150, 200, 250 mg/kg) did not modify the latency time compared with Veh ($p > 0.05$). The control group (Veh) was not able to protect the mice from death; in comparison, DZP protected 100% of the mice, MmAE (50 mg/kg) had 50% mortality protection, MmAE (100 mg/kg) had 75% mortality protection, MmAE (150 mg/kg) had 37.5% mortality protection, MmAE (200 mg/kg) had 87.5% mortality protection, and MmAE (250 mg/kg) had 50% mortality protection (Table 3). Figure 15 shows that DZP (1 mg/kg) significantly reduced the number of tonic seizures, clonic seizures, and total seizures induced by PTZ compared to Veh ($p < 0.05$). Similarly, F3

(100 mg/kg), F4-10 (100 mg/kg) and F14 (100 mg/kg) significantly reduced the number of tonic seizures, clonic seizures, and total seizures induced by PTZ compared to Veh ($p < 0.05$). DZP (1 mg/kg) increased seizure latency time compared to Veh ($p < 0.05$). F3 (100 mg/kg), F4-10 (100 mg/kg), and F14 (100 mg/kg) did not modify seizure latency time compared to Veh ($p > 0.05$). The control group (Veh) was not able to protect the mice from death; in comparison DZP protected 100% of the mice, F3 (100 mg/kg) had 100% mortality protection, F4-10 (100 mg/kg) had 83.33% mortality protection, and F14 (10 mg/kg) had 100% mortality protection (Table 4).

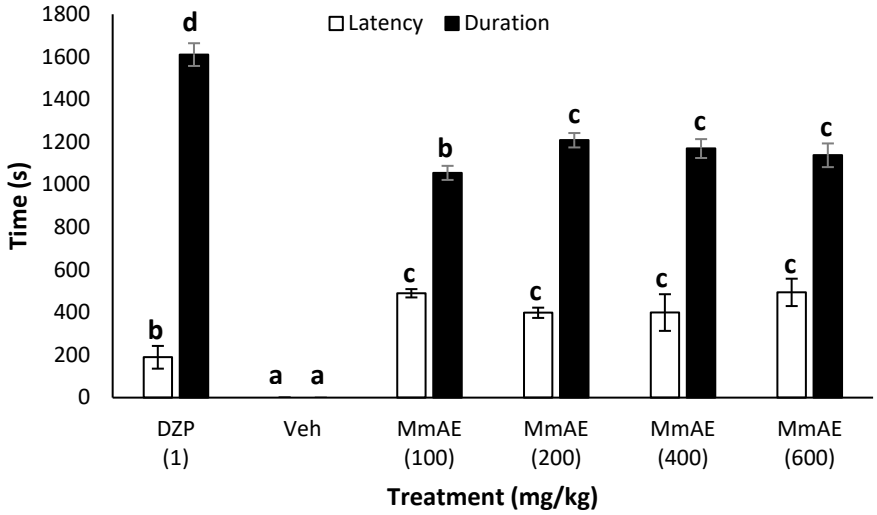


Figure 12. Effect of oral administration of the acetonic extract of *M. mexicana* A. Juss. (MmAE) on the latency and pentobarbital-induced sleep time of CD−1 mice exposed to the PBTt. Note: Compared with the Veh group, a = $p > 0.05$; b, c, d = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. DZP, diazepam; Veh, vehicle (100 µL/10 g); MmAE, acetonic extract of *M. mexicana* A. Juss.

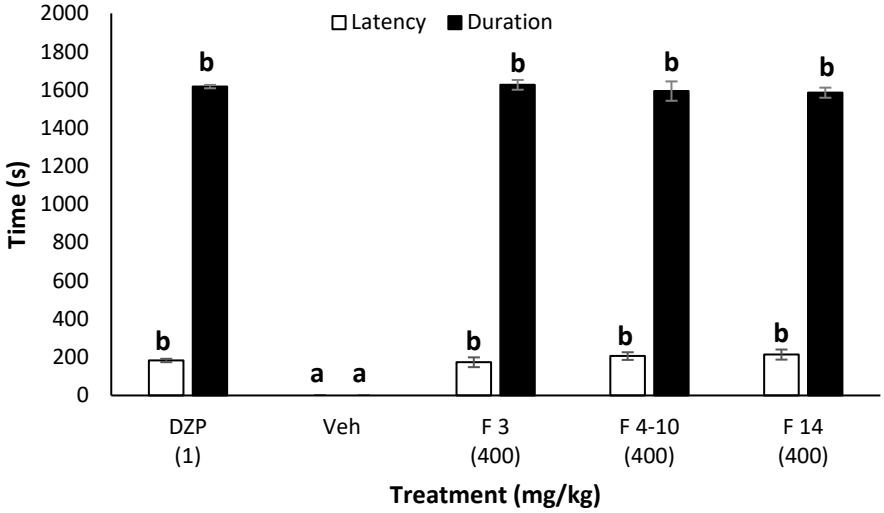


Figure 13. Effect of oral administration of three fractions of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the latency and the pentobarbital-induced sleep time of CD−1 mice exposed to the PBTt. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. DZP, diazepam; Veh, vehicle (100 µL/10 g).

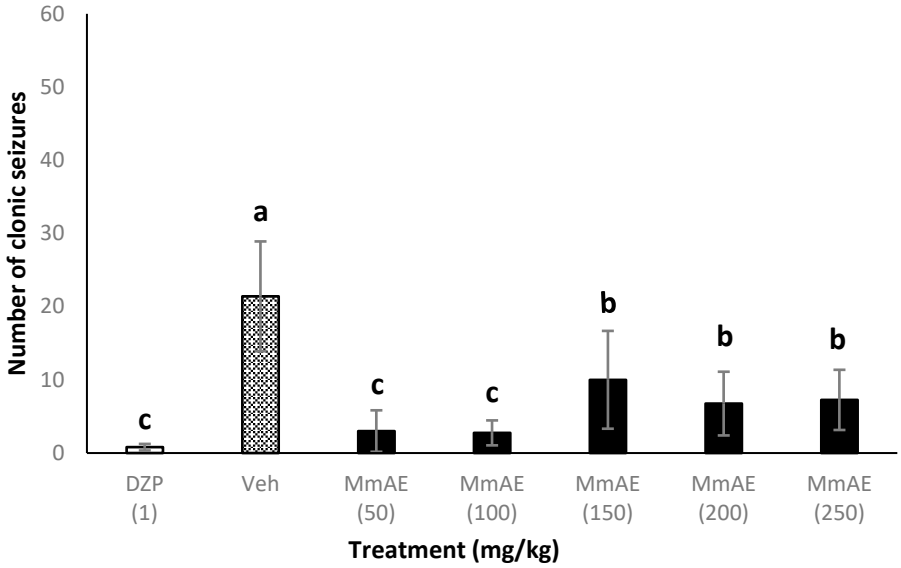


Figure 14. Effect of oral administration of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the number of clonic seizures in CD−1 mice exposed to PTZt. Note: Compared with the Veh group, a = $p > 0.05$; b = $p < 0.05$, c = $p < 0.001$ with ANOVA followed by Tukey's test (mean ± S.D.) with $n = 8$. DZP, diazepam; Veh, vehicle (100 µL/10 g); MmAE, acetonic extract of *M. mexicana* A. Juss.

Table 3. Anticonvulsant effect of acetonic extract of *M. mexicana* A. Juss. on PTZ-induced seizures in CD−1 mice.

Treatment (mg/kg)	Latency of Convulsions (s)	Mortality Protection (%)
Veh (100 µL/10 g)	54.00 ± 6.22 [a]	0.00
DZP (1.0)	131.75 ± 7.68 [b]	100.00
MmAE (50)	55.00 ± 2.83 [a]	50.00
MmAE (100)	54.50 ± 3.39 [a]	75.00
MmAE (150)	49.60 ± 5.55 [a]	37.50
MmAE (200)	53.20 ± 5.45 [a]	87.50
MmAE (250)	52.50 ± 6.45 [a]	50.00

Data presented as the mean ± S. D. with $n = 8$. a = $p > 0.05$, b = $p < 0.001$ compared to vehicle using ANOVA and *post hoc* Tukey's test. Veh, vehicle, DZP, diazepam; MmAE, acetonic extract of *M. mexicana* A. Juss.

Table 4. Anticonvulsant effect of three fractions of acetonic extract of *M. mexicana* A. Juss. on PTZ-induced seizures in CD−1 mice.

Treatment (mg/kg)	Latency of Convulsions (s)	Mortality Protection (%)
DZP (1.0)	131.75 ± 7.68 [b]	100.00
Veh (100 µL/10 g)	55.00 ± 05.45 [a]	0
F3 (100)	64.00 ± 10.84 [a]	100.00
F4-10 (100)	66.16 ± 03.25 [a]	83.33
F14 (100)	71.66 ± 11.46 [a]	100.00

Data presented as the mean ± S. D. with $n = 8$. a = $p > 0.05$, b = $p < 0.001$ compared to vehicle using ANOVA and *post hoc* Tukey's test. Veh, vehicle, DZP, diazepam; MmAE, acetonic extract of *M. mexicana* A. Juss.

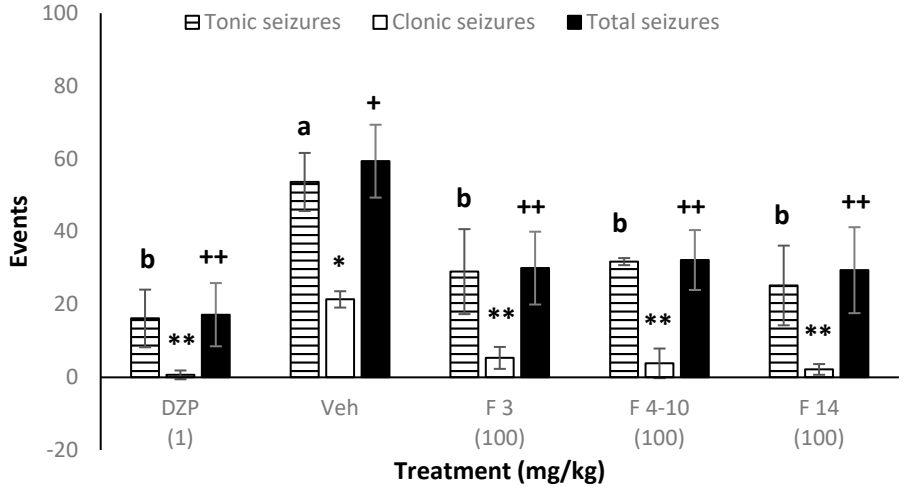


Figure 15. Effect of oral administration of three fractions of acetonic extract of *M. mexicana* A. Juss. (MmAE) on the number of clonic seizures in CD−1 mice exposed to PTZt. Note: Compared with the Veh group, a, *, + = $p > 0.05$; b, **, ++ = $p < 0.001$ with ANOVA followed by Tukey's *post hoc* test (mean ± S.D.). DZP, diazepam; Veh, vehicle (100 µL/10 g).

4. Discussion

Although there are reports in traditional Mexican medicine that *M. mexicana* A. Juss. is used by people of the REBIOSH to treat nervous disorders, this species had not been evaluated for its activity in the central nervous system (CNS). In this work, we investigated for the first time the effects on the CNS of an extract (MmAE) of the leaves of this plant and three fractions (F3, F4-10, and F14) in CD−1 mice using different neuropharmacological models. We also performed the first phytochemical profile of this plant species. Several studies have been conducted with polar extracts and compounds isolated from plants of the Malpighiaceae family and have shown CNS-level anxiolytic, antidepressant, hypnotic, anticonvulsant, and antischizophrenic effects in several pharmacological tests [4,17,32–37]. Some chemical compounds have been proposed to be responsible for these pharmacological effects.

Phytochemical profile of *M. mexicana* A. Juss. There are no previous reports on the phytochemistry of *M. mexicana*. However, there are some phytochemical studies of *Malpighia emarginata* DC. That report the presence of ascorbic acid, carotenoids (β-carotene), anthocyanins and flavonoids (rutin, quercetin, kaempferol, and their glycosylated versions) [38], polyphenols, anthocyanins, hydroxycinnamoyl derivatives (caffeoyl hexoside, dihydrocaffeoylquinic acid, and coumaroyl hexoside) [39], and norfriedelins [40].

In the present study, MmAE and F-14 were analyzed by HPLC, which showed evidence of compounds of the flavonoid group; comparison with standards allowed these compounds to be identified as quercetin 3-*O*-rutinoside (**1**), kaempferol 3-*O*-glucoside (**2**), luteolin 7-*O*-glucoside (**3**), quercetin (**4**), and kaempferol (**5**) in MmAE, while compounds (**1**), (**2**), and (**3**) were also identified in F14. In the present study, F3 and F4-10 were analyzed by GC-MS. In F3, α-tocopherol (26.5%) was identified, which is an antioxidant compound that is essential to prevent the establishment of several neurodegenerative diseases [41]. Phytol is also present (13.9%), which has been found in several reports to have neuropharmacological effects [42]. GC-MS analysis of F4-10 identified β-tocopherol (27.2%), which is an antioxidant and anti-inflammatory compound [43], phytol (10.7%), and β-sitosterol (10.7%), which is a compound that prevents several neurodegenerative diseases [44]. Some of the compounds identified in the MmAE extract and fractions F3, F4-10, and F14 have already been reported to have effects on the CNS, and even the mechanism of action is already known to exert some of the effects found here, such as antidepressant, anxiolytic, sedative, hypnotic, and anticonvulsant effects. We propose that the pharmacological effect found in MmAE, F3, F4-10, and F-14 in the different neuropharmacological models is due

to the presence of these compounds, and the mechanism of action may be similar to those reported by other working groups.

Neuropharmacological assays. *Antidepressant effect.* In the present work, MmAE, F3, F4-10, and F14 showed an antidepressant effect in the FST by reducing immobility time. A study of a plant of the same family, *Byrsonima crassifolia*, showed that a methanolic extract that was standardized in flavonoid concentration presented an antidepressant effect in the FST and determined that the compounds responsible for the antidepressant effect were quercetin, quercetin 3-*O*-xyloside, and rutin [34]. On the other hand, a methanolic extract of *Heteropterys cotinifolia* (Malpighiaceae) was shown to have a dose-dependent antidepressant effect without reduction of spontaneous motor activity; the main compounds in this extract to which this pharmacological effect is attributed were chlorogenic acid and rutin [4]. A polyphenol-rich fraction of *Origanum majorana* L. containing luteolin-7-*O*-glucoside, kaempferol-3-*O*-glucuronic acid, kaempferol-3-*O*-pentose, quercetin, and rutin presented an antidepressant effect in the forced swimming test and tail suspension test [45]. A study by Foudah et al., 2022, showed that rutine administration (80 mg/kg) to rats induced an antidepressant effect, and this effect was mediated by increased serotonin, norepinephrine, and dopamine levels in the cortical and hippocampal regions [46]. Likewise, a methanolic extract of *Heteropterys brachiata* (Malpighiaceae) induced an antidepressant effect in the FST, and the main compounds identified in this extract were chlorogenic acid and chlorogenic acid methyl ester [17]. A recent study reported that kaempferol produces an antidepressant effect by increasing the expression of Sirt3 in the hippocampus, the main mitochondrial deacetylase, and causing the subsequent activation of mitochondrial antioxidants [47]. Another study reported that phytol has an antidepressant effect [48]. The possible mechanism of action of MmAE and F3, F4-10, and F14 may be by regulating the concentration of serotonin and the monoamines norepinephrine and dopamine. In F3, the best effect occurred with the lowest dose, and the effect decreased with higher doses. Some drugs may present a pharmacological effect that does not correspond to a dose-dependent response (sigmoidal profile), called a dose-response relationship of biphasic nature, where the response is plotted as an inverted U, with low doses induce a favorable pharmacological effect and with increasing doses, the effect is lower and even the opposite effect may occur. This type of response is called a paradoxical response [49–51]. In F3 we identified the presence of phytol, a compound with antidepressant activity and which has also been reported to have a biphasic pharmacological behavior; this is why the best effect was observed at the lowest dose [48,52].

Anxiolytic effect. Here, MmAE exerted a dose-dependent anxiolytic effect in the EPM, but the F3, F4-10, and F14 fractions did not. When obtaining the three fractions that are chemically simpler, the compounds were separated according to their polarity and the anxiolytic effect is lost, which indicates that the anxiolytic effect of MmAE in EPM is due to a pharmacological synergy of the compounds present in it. In a previous study, the methanolic extract of *Galphimia glauca* (Malpighiaceae) leaves contained nor-friedelanes [33,37,53] and flavonoids, compounds which have been shown to have an anxiolytic effect in the EPM [36,54]. A methanolic extract of *Heteropterys brachiata* (Malpighiaceae) leaves had anxiolytic effect in the EPM and the chemical compounds identified were hydroxycinnamic acids and triterpenes [17]. A standardized fraction of flavonoids from *Tilia americana* was evaluated for anxiolytic activity with serotonergic drugs; the fraction contained the flavonols tyliroside, quercetin glycoside, quercitrin, rutin, and kaempferol [55]. In a recent study, kaempferol-3-*O*-β-D-glucoside, an anxiolytic flavonoid present in *Brassica oleracea* L., was isolated, characterized, and quantified [56]. Of the flavonoids identified in this study, quercetin-3-*O*-rutinoside (rutin) is reported to exert an anxiolytic effect on the basolateral amygdala, and this effect is mediated through modulation of $GABA_A$ receptor conductance [57]. Furthermore, Perez-Ortega et al., 2017, reported that quercetin-3-*O*-rutoside (rutin) and kaempferol 3-*O*-glucoside exert an anxiolytic and sedative effect through the modulation of $5\text{-}HT_{A1}$ receptors [58]. A study was recently published showing that quercetin induces anxiolytic effects through interaction with GABA receptors with

their α2, β1, and β2 subunits [59]. Grundmann et al., 2009, reported that kaempferol isolated from Apocynum venetum leaves showed an anxiolytic effect in the EPM model through GABAergic modulation [60]. Phytol presented an anxiolytic effect in the EPM model, light–dark test, and marble-burying model through modulation of GABAergic neurotransmission [61].

Sedative effect. The open field test is able to evaluate the locomotor effects and the exploratory activity of drugs in mice. The animal, in a new environment, has a tendency to explore it, despite stress and conflict. The test of spontaneous locomotor activity is used as a general parameter to study the central action of a drug [62]. A decrease in locomotor activity means that the drug has a depressant effect on the CNS in the animal under study, i.e., a motor change occurs that may interfere with other behavioral tests performed on the same animal [27]. In the present work, DZP (1 mg/kg) did not modify the parameters of total crossings and rearings compared to Veh. It is known that benzodiazepines induce a sedative effect, which is dose-dependent; many studies have reported that DZP (2 mg/kg) significantly decreases the number of total OFT crossovers and the number of rearings compared to the control group [63–65]. MmAE, F3, F4-10, and F14 showed a sedative effect in the OFT by reducing the total crossings, but without modifying the rearings. It has been shown that plants belonging to the Malpighiaceae family have compounds that induce a sedative effect similar to the one found in the present work; from the methanolic extract of *Galphimia glauca* (Malpighiaceae), several nor-seco-triterpenes with sedative effects were isolated [54]. The ethanolic extract of *Heteropterys glabra*, a species that also belongs to Malpighiaceae, induced a sedative effect at a dose of 350 mg/kg in DBA/2J mice [35]. Jeon et al., 2015, reported that the administration of β-amyrin induced a sedative effect in the OFT model by decreasing locomotor activity; this effect was due to the modulation of GABAergic neurotransmission [66]. β-Sitosterol significantly upregulated the amount of protein-level expression of Glu decarboxylase-65 (GAD65) and the α1-subunit of $GABA_A$ receptors in the hypothalamus of mice, not affecting GAD67 or γ2 subunits [67]. Phytol (75 mg/kg) altered the rotational performance of mice in the motor activity test. The authors suggest that phytol interacts with the $GABA_A$ receptor, probably with receptor subtypes that mediate the effects of benzodiazepines [61]. These results are related to the results found in the present study, where MmAE extract and fractions F3, F4-10, and F14 decreased motor activity by decreasing the number of total crossings in the OFT model compared to the vehicle group; this result may be due to the presence, at least in part, of β-amyrin, β-sitosterol, and phytol. And the mechanism of action is by regulating GABAergic neurotransmission. In addition, a study found that quercetin-3-*O*-rutoside (rutin) and kaempferol 3-*O*-glucoside exert a sedative effect through the modulation of $5\text{-}HT_{A1}$ receptors [58], which may be an alternate mechanism for the MmAE extract to induce the sedative effect.

Hypnotic effect. In the present work, MmAE, F3, F4-10, and F14 induced a hypnotic effect by increasing the pentobarbital-induced sleep time. A methanolic extract of *Galphimia glauca* (Malpighiaceae) showed a hypnotic effect in the PBTt [32], and nor-friedelanes, which have a hypnotic effect [33], were isolated. One study indicated that administration of β-amyrin was able to significantly decrease sleep latency and increase sleep duration in the pentobarbital-induced sleep model compared to the control group. This effect is due to the fact that β-amyrin is able to activate the GABAergic neurotransmitter system [66]. It was determined that β-sitosterol induces a hypnotic effect through modulation of the GABAergic system, as β-sitosterol significantly upregulated the amount of protein-level expression of Glu decarboxylase-65 (GAD65) and α1 subunits of GABAA receptors in the hypothalamus of mice, not affecting GAD67 or γ2 subunits [67]. In another study, the oral administration of β-sitosterol to mice for 7 days induced a reduction in sleep latency time and increased the duration of sleep time significantly compared to the control group in the pentobarbital-induced sleep model. In addition, administration of β-sitosterol increased the expression of melatonin receptor MT1 and MT2 mRNA in the brain cortex (1.5- and 3.8-fold, respectively) [68]. In the same study, it was observed that β-sitosterol

significantly increased the number of alpha waves (450%) in an electroencephalographic recording, which was related to a reduction in the sleep latency period. Phytol (75 mg/kg) impaired the rota-rod performance of mice in motor activity test. The authors suppose that phytol interacts with $GABA_A$ receptor, probably at the receptor subtypes that mediate benzodiazepines effects [61].

Anticonvulsant effect. In the present study, MmAE (50, 100, 150, 200, 250 mg/kg) had different percentages of protection against mortality compared to Veh. MmAE (50 and 100 mg/kg) significantly decreased the number of clonic seizures ($p < 0.001$), and MmAE (150, 200 and 250 mg/kg) also decreased the number of clonic seizures with a significance of $p < 0.05$. The fact that lower doses showed a better effect than higher doses in decreasing the number of clonic seizures may be due to a biphasic pharmacological behavior [49,52]. F3 (100 mg/kg), F4-10 (100 mg/kg), and F14 (100 mg/kg) were able to decrease the number of tonic seizures, clonic seizures, and total seizures significantly compared to Veh, and these results were statistically similar to those obtained with DZP (1 mg/kg), evidencing that the fractions, despite having compounds of different chemical nature, induced an anticonvulsant effect in the PTZt. The protective effect against mortality obtained with F3 (100 mg/kg) was 100%; F4-10 (100 mg/kg) had a mortality protection of 83.33% and F4-10 (100 mg/kg) had a mortality protection of 100%. The anticonvulsant results obtained with the fractions were better than those obtained with the MmAE extract.

The flavonoids quercetin and quercetin glycoside are known to have neuroprotective effects against a wide range of neurological disorders including Alzheimer's disease, Parkinson's disease, traumatic brain injury, and epilepsy [69]. Likewise, a methanolic extract of *Galphimia glauca* (Malpighiaceae) showed anticonvulsant effect in the strychnine-induced convulsions model and PTZt [32]. A methanolic extract of *Heteropterys brachiata* (Malpighiaceae) leaves had an anticonvulsant effect in the PTZt, and the chemical compounds identified were hydroxycinnamic acids and triterpenes [17]. Another study showed that the methanolic extract of *Tilia americana* var. *mexicana* had an anticonvulsant effect in the PTZt; the pharmacological effect was attributed to the presence in the extract of the flavonoids quercetin, rutin, and isoquercetin [70]. A study evaluated the anticonvulsant effects of quercetin and rutin in different models of induced seizures and found that they had a short-term anticonvulsant potential at low doses, and when used in combination with anticonvulsant drugs, they did not modify the action of these drugs and did not present additional side effects [71]. On the other hand, a recent study evaluated the anticonvulsant effect of chronic quercetin administration in the penicillin-induced seizure model found that low doses of quercetin had a better anticonvulsant effect by increasing the latency time [72]. In a recent study evaluating the anticonvulsant effect of quercetin administration to mice in the PTZ-induced seizure model, quercetin was found to increase the seizure threshold by reducing the inflammatory response and oxidative stress in the prefrontal cortex [73]. One study showed that phytol pretreatment (25, 50, and 75 mg/kg) was able to increase the latency of the first seizure and decrease the percentage of seizures; phytol protected animals against pilocarpine-induced status epilepticus and decreased the mortality rate [42]. It has been proposed that the mechanism of action for the anticonvulsant effect of phytol is through the elevation of GABA levels in the CNS, and one way to achieve this is to inhibit GABA-degrading enzymes. In this sense, a study showed evidence that phytol acts as an irreversible inhibitor of the enzyme succinic semialdehyde dehydrogenase (SSADH). GABA degradation is achieved in a two-step reaction; GABA is transaminated by GABA-transaminase (GABA-T) to succinic semialdehyde (SSA), which is then converted to succinate by the enzyme succinic semialdehyde dehydrogenase (SSADH). Succinate thus enters the tricarboxylic acid cycle [74]. It has been reported that α-amyrin and β-amyrin triterpenes induce a dose-dependent anticonvulsant effect in the PTZt model, the mechanism of action being through elevation of the concentration of excitatory and inhibitory amino acid neurotransmitters in the basal ganglia and hippocampus [75]. In a recent study, it was confirmed that quercetin increased the seizure threshold and total antioxidant capacity; quercetin decreased levels of malondialdehyde as well as gene expression of TNF-

α, NLRP3, IL-1β, and iNOS in the prefrontal cortex at the time of seizure induction. It was suggested that the anticonvulsant effect of quercetin in PTZ-induced seizures in mice may be due to the reduction of inflammatory responses and oxidative stress in the prefrontal cortex [73].

GC-MS analysis of F3 identified α-tocopherol (26.5%), which has been reported to be the most important lipophilic radical scavenging antioxidant [41]. It has also been shown that oxidative stress is one of the causes of the establishment of several neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis, among others, because the proper and normal functioning of the central nervous system depends entirely on the integrity of the nerve cells [76]. Phytol was another compound detected in F3 (13.9%). Several studies show that phytol is capable of presenting neuropharmacological effects. For example, a study carried out by Costa, Ferreira, De Sousa, Jordan, and Freitas [42] found that different doses of phytol (25, 50, 75 mg/kg, *i.p.*) delayed the latency of the first seizure and decrease the percentage of seizures, in addition to protecting mice from death in the pilocarpine-induced seizure model. These results are similar to those found in the present work, since the MmAE extract and its fractions decrease the number of seizures. In another study, it was found that phytol (25, 50, 100, 200 mg/kg, *i.p.*) was able to induce an antinociceptive effect or an antioxidant effect in three different models [77]. It was reported that phytol is used in anxiolytic and antidepressant formulations [78]; these results strengthen the proposal that the anxiolytic and antidepressant effects of MmAE extract may be due to the presence of phytol. Similarly, Costa, et al. [61], reported anxiolytic and sedative activity of phytol (25, 50, 75 mg/kg, *i.p.*) and proposed a GABAergic type mechanism of action. We also detected β-amyrin in F3 (12.3%), which is a triterpene for which neurological effects have been reported, such as sleep regulation. Other studies suggest that β-amyrin could enhance the total sleeping behavior in a pentobarbital-induced sleeping model via the activation of GABAergic neurotransmitter system through GABA content in the brain [66], improve memory [79], have and antinociceptive effect [80], and improve object recognition in animals with Alzheimer's disease while ameliorating β-amyloid-induced neurogenesis impairment [81]. In the present work, MmAE extract and its fractions also modified sleep by inducing a hypnotic effect, and it is highly probable that β-amyrin is one of the compounds contributing to this pharmacological effect.

GC-MS analysis detected β-tocopherol (27.2%) in F4-10, which has been reported to have important antioxidant and anti-inflammatory effects [82]. Many American physicians prescribe the use of tocopherol for the treatment of Alzheimer's disease, as recommended by the American Academy of Neurology and the American Psychiatric Association [83]. Other compounds determined in this fraction include phytol (10.7%), whose involvement in the nervous system has already been mentioned, and β-sitosterol (9.6%), which is part of the group of phytosterols that (unlike cholesterol) have been reported to cross the blood–brain barrier and accumulate in the brain [84]. This leads to the replacement of brain cholesterol, resulting in improvements in several neurodegenerative diseases such as Alzheimer's disease [85]. In a recent study, β-sitosterol was found to reduce anxiety, the effects of restraint stress, contextual fear memory, and c-Fos activation in the prefrontal cortex and dentate gyrus. In addition, synergistic anxiolysis is observed when sub-effective doses of β-sitosterol are combined with the SSRI fluoxetine [86]. Also present in F4-10 is β-amyrin (2.9%), whose effects on the nervous system have already been mentioned. In the future, it would be desirable to evaluate the pharmacological effects of the isolated chemical compounds individually and establish their possible mechanisms of action.

In the present work, it was evidenced that an acetonic extract of leaves of *Malpighia mexicana* A. Juss. and its fractions have antidepressant, anxiolytic, sedative, hypnotic, and anticonvulsant effects. This allows us to propose this species as a good source of active compounds, some of which could represent alternatives for the treatment of some mental and neurological disorders. *M. mexicana* A. Juss. is a viable plant for cultivation. The plant is drought-tolerant and can grow in nutrient-deficient soil. The species produces fruits

with highly viable seeds (90% viability) [87]. The main propagation method is by seeds, but vegetative propagation by cuttings (which retains the parental genotype) has been achieved with great success (97.5% survival) [88]. Because of this, *Malpighia mexicana* A. Juss. is as a sustainable source of active compounds with effects on the central nervous system.

5. Conclusions

This study represents the first report of the phytochemical and pharmacological effect of *M. mexicana* A. Juss. on the central nervous system in CD−1 mice. HPLC analysis of the acetonic extract of *M. mexicana* A. Juss leaves (MmAE) and F14 allowed the identification of flavonoid-type compounds such as quercetin 3-*O*-rutinoside (**1**), kaempferol 3-*O*-glucoside (**2**), luteolin 7-*O*-glucoside (**3**), quercetin (**4**), and kaempferol (**5**). GC-MS analysis of F3 and F4-10 determined the presence of vitamins such as tocopherol, diterpenes such as phytol, phytosterols such as β-sitosterol, and triterpenes such as α- amyrin and β-amyrin. MmAE showed an antidepressant effect in the FST, an anxiolytic effect in the EPM, a sedative effect in the OFT, a hypnotic effect in the PBTt, and an anticonvulsant effect in the PTZt. The fractions F3, F4-10, and F14 showed an antidepressant effect in FST; F3, F4-10, and F14 presented a sedative effect in the OFT, a hypnotic effect in the PBTt, and an anticonvulsant effect in the PTZt, but F3, F4-10, and F14 did not present an anxiolytic effect in the EPM. MmAE and its fractions contain several compounds such as vitamins, phytosterols, terpenes, and flavonoids, which may be responsible for the neuropharmacological effects found in this study. This research requires further studies to clarify the mechanism of action of the compounds identified.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/scipharm91040047/s1, Figure S1: UPLC chromatogram and UV spectra of F14; Figure S2: MS data of peak 1 identified in F3; Figure S3: MS data of peak 2 identified in F3; Figure S4: MS data of peak 3 identified in F3; Figure S5: MS data of peak 4 identified in F3; Figure S6: MS data of peak 5 identified in F3; Figure S7: MS data of peak 6 identified in F3; Figure S8: MS data of peak 7 identified in F3; Figure S9: MS data of peak 8 identified in F3; Figure S10: MS data of peak 9 identified in F3; Figure S11: MS data of peak 1 identified in F4-10; Figure S12: MS data of peak 2 identified in F4-10; Figure S13: MS data of peak 3 identified in F4-10; Figure S14: MS data of peak 4 identified in F4-10; Figure S15: MS data of peak 5 identified in F4-10; Figure S16: Irwin test of the acetone extract of *M. mexicana* A. Juss. leaves (MmAE).

Author Contributions: Conceptualization, D.A.-M., D.O.S.-S., C.S.-L., A.Z. and M.G.-C.; Investigation, D.A.-M., D.O.S.-S., C.S.-L., A.Z., J.M.R.-G., V.L.P. and M.G.-C.; Methodology, D.A.-M., D.O.S.-S., C.S.-L., F.I.B.-M., R.A.-V. and D.L.M.-F.; Resources, D.A.-M., D.O.S.-S., C.S.-L., A.Z., Ó.D., R.F.-B. and M.G.-C.; Supervision, D.A.-M., D.O.S.-S., A.Z. and M.G.-C.; Validation, D.A.-M., D.O.S.-S., C.S.-L., A.Z., R.A.-V. and M.G.-C.; Writing—original draft, D.A.-M. and D.O.S.-S.; Writing—review & editing, D.A.-M., D.O.S.-S., C.S.-L., A.Z., R.A.-V. and M.G.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Ethics Committee of Mexican Institute of Social Security (R-2023-1702-008, 28 July 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available upon reasonable request.

Acknowledgments: The authors would like to thank M.C. Gabriel Flores, curator of the HUMO Herbarium of CIByC-UAEM, for the collection and identification of the plant material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bárcenas-López, L.Y.; Montaño-Arias, S.A.; López-Sandoval, J.A.; González, A.; Rubí-Arriaga, M.; Vargas, G. Anatomía foliar de *Malpighia mexicana* (Malpighiaceae). *Acta Bot. Mex.* **2019**, *126*, 1–12. [CrossRef]

2. Maldonado-Peralta, M.Á.; García-de los Santos, G.; García-Nava, J.R.; Corona-Torres, T.; Cetina-Alcalá, V.M.; Ramírez-Herrera, C. Calidad morfológica de frutos y endocarpios del nanche rojo (*Malpighia mexicana*, Malpighiaceae). *Acta Botánica Mex.* **2016**, *117*, 37–46. [CrossRef]
3. Dorado, O. *Programa de Conservación y Manejo Reserva de la Biosfera Sierra de Huautla*, 1st ed.; Comisión Natural de Áreas Naturales Protegidas: México City, México, 2005.
4. Huerta-Reyes, M.; Zamilpa, A.; Álvarez-Chimal, R.; Luna-Manzanares, J.; León-Velasco, M.E.; Aguilar-Rojas, A.; Jiménez-Estrada, M.; Campos-Lara, M.G. Heteropterys cotinifolia: A neuropharmacological and phytochemical approach with possible taxonomic implications. *Sci. World J.* **2013**, *2013*, 870468. [CrossRef] [PubMed]
5. BDMTM. Ajuaxocotl *Malpighia mexicana* Juss.—Malpighiaceae. Available online: http://www.medicinatradicionalmexicana.unam.mx/apmtm/termino.php?l=3&t=malpighia-mexicana (accessed on 17 March 2023).
6. Calixto, J.B. The role of natural products in modern drug discovery. *An. Acad. Bras. Ciências* **2019**, *91*, 1–7. [CrossRef]
7. Opio, J.N.; Munn, Z.; Aromataris, E. Prevalence of Mental Disorders in Uganda: A Systematic Review and Meta-Analysis. *Psychiatr. Q.* **2022**, *93*, 199–226. [CrossRef]
8. Chellappa, S.L.; Aeschbach, D. Sleep and anxiety: From mechanisms to interventions. *Sleep Med. Rev.* **2022**, *61*, 101583. [CrossRef]
9. Bhatt, S.; Devadoss, T.; Manjula, S.N.; Rajangam, J. 5-HT(3) receptor antagonism a potential therapeutic approach for the treatment of depression and other disorders. *Curr. Neuropharmacol.* **2021**, *19*, 1545–1559. [CrossRef]
10. Ziobrowski, H.N.; Kennedy, C.J.; Ustun, B.; House, S.L.; Beaudoin, F.L.; An, X.; Zeng, D.; Bollen, K.A.; Petukhova, M.; Sampson, N.A.; et al. Development and Validation of a Model to Predict Posttraumatic Stress Disorder and Major Depression After a Motor Vehicle Collision. *JAMA Psychiatry* **2021**, *78*, 1228–1237. [CrossRef]
11. Choi, K.W.; Kim, Y.K.; Jeon, H.J. Comorbid Anxiety and Depression: Clinical and Conceptual Consideration and Transdiagnostic Treatment. *Adv. Exp. Med. Biol.* **2020**, *1191*, 219–235. [CrossRef]
12. Thijs, R.D.; Surges, R.; O'Brien, T.J.; Sander, J.W. Epilepsy in adults. *Lancet* **2019**, *393*, 689–701. [CrossRef]
13. Leichsenring, F.; Steinert, C.; Rabung, S.; Ioannidis, J.P.A. The efficacy of psychotherapies and pharmacotherapies for mental disorders in adults: An umbrella review and meta-analytic evaluation of recent meta-analyses. *World Psychiatry* **2022**, *21*, 133–145. [CrossRef] [PubMed]
14. Davies, P.; Ijaz, S.; Williams, C.J.; Kessler, D.; Lewis, G.; Wiles, N. Pharmacological interventions for treatment-resistant depression in adults. *Cochrane Database Syst. Rev.* **2019**, *12*, Cd010557. [CrossRef] [PubMed]
15. Pham Nguyen, T.P.; Soprano, S.E.; Hennessy, S.; Brensinger, C.M.; Bilker, W.B.; Miano, T.A.; Acton, E.K.; Horn, J.R.; Chung, S.P.; Dublin, S.; et al. Population-based signals of benzodiazepine drug interactions associated with unintentional traumatic injury. *J. Psychiatr. Res.* **2022**, *151*, 299–303. [CrossRef]
16. Friedman, J.H. Movement disorders induced by psychiatric drugs that do not block dopamine receptors. *Park. Relat. Disord.* **2020**, *79*, 60–64. [CrossRef] [PubMed]
17. Huerta-Reyes, M.; Herrera-Ruiz, M.; González-Cortazar, M.; Zamilpa, A.; León, E.; Reyes-Chilpa, R.; Aguilar-Rojas, A.; Tortoriello, J. Neuropharmacological in vivo effects and phytochemical profile of the extract from the aerial parts of *Heteropterys brachiata* (L.) DC. (Malpighiaceae). *J. Ethnopharmacol.* **2013**, *146*, 311–317. [CrossRef]
18. Gallegos-García, A.J.; Lobato-García, C.E.; González-Cortazar, M.; Herrera-Ruiz, M.; Zamilpa, A.; Álvarez-Fitz, P.; Pérez-García, M.D.; López-Rodríguez, R.; Ble-González, E.A.; Medrano-Sánchez, E.J.; et al. Preliminary Phytochemical Profile and Bioactivity of Inga jinicuil Schltdl & Cham. ex G. Don. *Plants* **2022**, *11*, 794. [CrossRef]
19. Adams, R.P. (Ed.) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007; p. 804.
20. Kallman, M.J. Despair Swim Test. In *Drug Discovery and Evaluation: Pharmacological Assays*, 4th ed.; Hock, F.J., Ed.; Springer International Publishing: Berlin/Heidelberg, Germany, 2016; pp. 1450–1453.
21. Vogel, H.G.; Vogel, W.H. (Eds.) Antidepressant activity. In *Drug Discovery and Evaluation: Pharmacological Assays*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 302–303.
22. Kallman, M.J. Elevated Plus Maze Test. In *Drug Discovery and Evaluation: Pharmacological Assays*, 4th ed.; Hock, F.J., Ed.; Springer International Publishing: Berlin/Heidelberg, Germany, 2016; pp. 1127–1130.
23. La-Vu, M.; Tobias, B.C.; Schuette, P.J.; Adhikari, A. To Approach or Avoid: An Introductory Overview of the Study of Anxiety Using Rodent Assays. *Front. Behav. Neurosci.* **2020**, *14*, 145. [CrossRef]
24. Walf, A.A.; Frye, C.A. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* **2007**, *2*, 322–328. [CrossRef]
25. Vogel, H.G.; Vogel, W.H. (Eds.) Open field test. In *Drug Discovery and Evaluation: Pharmacological Assays*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 206–208.
26. Kallman, M.J. Open Field Test. In *Drug Discovery and Evaluation: Pharmacological Assays*, 4th ed.; Hock, F.J., Ed.; Springer International Publishing: Berlin/Heidelberg, Germany, 2016; pp. 1044–1048.
27. Kraeuter, A.K.; Guest, P.C.; Sarnyai, Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-like Behavior. *Methods Mol. Biol.* **2019**, *1916*, 99–103. [CrossRef]
28. Kallman, M.J. Potentiation of Hexobarbital Sleeping Time. In *Drug Discovery and Evaluation: Pharmacological Assays*, 4th ed.; Hock, F.J., Ed.; Springer International Publishing: Berlin/Heidelberg, Germany, 2016; pp. 1307–1308.

29. Vogel, H.G.; Vogel, W.H. (Eds.) Potentiation of hexobarbital sleeping time. In *Drug Discovery and Evaluation: Pharmacological Assays*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 267–268.
30. Kallman, M.J. Anticonvulsant activity: Pentylenetetrazole metrazole-Induce Convulsions. In *Drug Discovery and Evaluation: Pharmacological Assays*, 4th ed.; Hock, F.J., Ed.; Springer International Publishing: Berlin/Heidelberg, Germany, 2016; pp. 1109–1111.
31. Vogel, H.G.; Vogel, W.H. (Eds.) Anticonvulsant activity: Pentylentetrazole (Metrazole) induced convulsions. In *Drug Discovery and Evaluation: Pharmacological Assays*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 227–228.
32. Tortoriello, J.; Lozoya, X. Effect of Galphimia glauca methanolic extract on neuropharmacological tests. *Planta Med.* **1992**, *58*, 234–236. [CrossRef]
33. Rios, M.Y.; Ortega, A.; Domínguez, B.; Déciga, M.; Rosa, V. Glaucacetalin E and galphimidin B from Galphimia glauca and their anxiolytic activity. *J. Ethnopharmacol.* **2020**, *259*, 112939. [CrossRef]
34. Herrera-Ruiz, M.; Zamilpa, A.; González-Cortazar, M.; Reyes-Chilpa, R.; León, E.; García, M.P.; Tortoriello, J.; Huerta-Reyes, M. Antidepressant effect and pharmacological evaluation of standardized extract of flavonoids from *Byrsonima crassifolia*. *Phytomedicine* **2011**, *18*, 1255–1261. [CrossRef] [PubMed]
35. Galietta, G.; Giuliani, G.; Loizzo, A.; Amat, A.G.; Fumagalli, E.; De Feo, V.; Quaranta, E.; Paladino, L.; Capasso, A. Neurophysiological studies of *Heteropteris glabra* Hok. & Arn. (Malpighiaceae) in DBA/2J mice. *J. Ethnopharmacol.* **2005**, *97*, 415–419. [CrossRef] [PubMed]
36. Herrera-Ruiz, M.; Jiménez-Ferrer, J.E.; De Lima, T.C.; Avilés-Montes, D.; Pérez-García, D.; González-Cortazar, M.; Tortoriello, J. Anxiolytic and antidepressant-like activity of a standardized extract from Galphimia glauca. *Phytomedicine* **2006**, *13*, 23–28. [CrossRef]
37. Avilés-Montes, D.; Herrera-Ruiz, M.; Román-Ramos, R.; Jiménez-Ferrer, E.; González-Cortazar, M.; Zamilpa, A.; Tortoriello, J. Pharmacological Interaction between Galphimine-A, a Natural AnxiolyticCompound and Gabaergic Drugs. *Int. J. Pharmacol.* **2015**, *11*, 944–955. [CrossRef]
38. Prakash, A.; Baskaran, R. Acerola, an untapped functional superfruit: A review on latest frontiers. *J. Food Sci. Technol.* **2018**, *55*, 3373–3384. [CrossRef] [PubMed]
39. Alvarez-Suarez, J.M.; Giampieri, F.; Gasparrini, M.; Mazzoni, L.; Santos-Buelga, C.; González-Paramás, A.M.; Forbes-Hernández, T.Y.; Afrin, S.; Páez-Watson, T.; Quiles, J.L.; et al. The protective effect of acerola (*Malpighia emarginata*) against oxidative damage in human dermal fibroblasts through the improvement of antioxidant enzyme activity and mitochondrial functionality. *Food Funct.* **2017**, *8*, 3250–3258. [CrossRef]
40. Liu, J.Q.; Peng, X.R.; Li, X.Y.; Li, T.Z.; Zhang, W.M.; Shi, L.; Han, J.; Qiu, M.H. Norfriedelins A-C with acetylcholinesterase inhibitory activity from acerola tree (*Malpighia emarginata*). *Org. Lett.* **2013**, *15*, 1580–1583. [CrossRef] [PubMed]
41. Blaner, W.S.; Shmarakov, I.O.; Traber, M.G. Vitamin A and Vitamin E: Will the Real Antioxidant Please Stand Up? *Annu. Rev. Nutr.* **2021**, *41*, 105–131. [CrossRef]
42. Costa, J.P.; Ferreira, P.B.; De Sousa, D.P.; Jordan, J.; Freitas, R.M. Anticonvulsant effect of phytol in a pilocarpine model in mice. *Neurosci. Lett.* **2012**, *523*, 115–118. [CrossRef]
43. Violi, F.; Nocella, C.; Loffredo, L.; Carnevale, R.; Pignatelli, P. Interventional study with vitamin E in cardiovascular disease and meta-analysis. *Free Radic. Biol. Med.* **2022**, *178*, 26–41. [CrossRef] [PubMed]
44. Khan, Z.; Nath, N.; Rauf, A.; Emran, T.B.; Mitra, S.; Islam, F.; Chandran, D.; Barua, J.; Khandaker, M.U.; Idris, A.M.; et al. Multifunctional roles and pharmacological potential of β-sitosterol: Emerging evidence toward clinical applications. *Chem. Biol. Interact.* **2022**, *365*, 110117. [CrossRef]
45. Amaghnouje, A.; Mechchate, H.; Es-Safi, I.; Boukhira, S.; Aliqahtani, A.S.; Noman, O.M.; Nasr, F.A.; Conte, R.; Calarco, A.; Bousta, D. Subacute Assessment of the Toxicity and Antidepressant-Like Effects of *Origanum Majorana* L. Polyphenols in Swiss Albino Mice. *Molecules* **2020**, *25*, 5653. [CrossRef] [PubMed]
46. Foudah, A.I.; Alqarni, M.H.; Alam, A.; Devi, S.; Salkini, M.A.; Alam, P. Rutin Improves Anxiety and Reserpine-Induced Depression in Rats. *Molecules* **2022**, *27*, 7313. [CrossRef]
47. Li, H.Y.; Wang, J.; Liang, L.F.; Shen, S.Y.; Li, W.; Chen, X.R.; Li, B.; Zhang, Y.Q.; Yu, J. Sirtuin 3 Plays a Critical Role in the Antidepressant- and Anxiolytic-like Effects of Kaempferol. *Antioxidants* **2022**, *11*, 1886. [CrossRef] [PubMed]
48. Pereira Costa, J.; da Silva Oliveira, J.; Mario Rezende Junior, L.; de Freitas, R.M. Phytol a Natural Diterpenoid with Pharmacological Applications on Central Nervous System: A Review. *Recent. Pat. Biotechnol.* **2014**, *8*, 194–205. [CrossRef]
49. Pérez-Davison, G.; Restrepo-Manrique, R.; Martínez-Sánchez, G. Hormesis: Antecedentes e Implicaciones en los Sistemas Biológicos. *Lat. Am. J. Pharm.* **2009**, *28*, 954–960.
50. Chirumbolo, S. Hormesis, resveratrol and plant-derived polyphenols: Some comments. *Hum. Exp. Toxicol.* **2011**, *30*, 2027–2030. [CrossRef]
51. Calabrese, E.J.; Mattson, M.P.; Calabrese, V. Dose response biology: The case of resveratrol. *Hum. Exp. Toxicol.* **2010**, *29*, 1034–1037. [CrossRef]
52. Calabrese, E.J.; Mattson, M.P. How does hormesis impact biology, toxicology, and medicine? *NPJ Aging Mech. Dis.* **2017**, *3*, 13. [CrossRef]

53. Romero-Cerecero, O.; Islas-Garduño, A.L.; Zamilpa, A.; Pérez-García, M.D.; Tortoriello, J. Therapeutic Effectiveness of Galphimia glauca in Young People with Social Anxiety Disorder: A Pilot Study. *Evid. Based Complement. Altern. Med.* **2018**, *2018*, 1716939. [CrossRef] [PubMed]
54. Ortíz, A.; Cardoso-Taketa, A.; Monroy, M.R.; Arellano, J.; Hernández, G.; Villarreal, M.L. Transformed cell suspension culture of Galphimia glauca producing sedative nor-friedelanes. *Planta Med.* **2010**, *76*, 386–392. [CrossRef] [PubMed]
55. Noguerón-Merino, M.C.; Jiménez-Ferrer, E.; Román-Ramos, R.; Zamilpa, A.; Tortoriello, J.; Herrera-Ruiz, M. Interactions of a standardized flavonoid fraction from Tilia americana with Serotoninergic drugs in elevated plus maze. *J. Ethnopharmacol.* **2015**, *164*, 319–327. [CrossRef] [PubMed]
56. Kaur, D.; Shri, R.; Kamboj, A. Bioactivity-directed isolation, characterization, and quantification of an anxiolytic flavonoid from Brassica oleracea L. *J. Food Biochem.* **2021**, *45*, e13608. [CrossRef]
57. Hernandez-Leon, A.; González-Trujano, M.E.; Fernández-Guasti, A. The anxiolytic-like effect of rutin in rats involves GABAA receptors in the basolateral amygdala. *Behav. Pharmacol.* **2017**, *28*, 303–312. [CrossRef]
58. Pérez-Ortega, G.; Angeles-López, G.E.; Argueta-Villamar, A.; González-Trujano, M.E. Preclinical evidence of the anxiolytic and sedative-like activities of Tagetes erecta L. reinforces its ethnobotanical approach. *Biomed. Pharmacother.* **2017**, *93*, 383–390. [CrossRef]
59. Islam, M.S.; Hossain, R.; Ahmed, T.; Rahaman, M.M.; Al-Khafaji, K.; Khan, R.A.; Sarkar, C.; Bappi, M.H.; de Andrade, E.M.; Araújo, I.M.; et al. Anxiolytic-like Effect of Quercetin Possibly through GABA Receptor Interaction Pathway: In Vivo and In Silico Studies. *Molecules* **2022**, *27*, 7149. [CrossRef]
60. Grundmann, O.; Nakajima, J.; Kamata, K.; Seo, S.; Butterweck, V. Kaempferol from the leaves of Apocynum venetum possesses anxiolytic activities in the elevated plus maze test in mice. *Phytomedicine* **2009**, *16*, 295–302. [CrossRef]
61. Costa, J.P.; de Oliveira, G.A.L.; de Almeida, A.A.C.; Islam, M.T.; de Sousa, D.P.; de Freitas, R.M. Anxiolytic-like effects of phytol: Possible involvement of GABAergic transmission. *Brain Res.* **2014**, *1547*, 34–42. [CrossRef]
62. Tatem, K.S.; Quinn, J.L.; Phadke, A.; Yu, Q.; Gordish-Dressman, H.; Nagaraju, K. Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. *J. Vis. Exp.* **2014**, e51785. [CrossRef]
63. Chaves, E.M.C.; Honório-Júnior, J.E.R.; Sousa, C.N.S.; Monteiro, V.S.; Nonato, D.T.T.; Dantas, L.P.; Lúcio, A.; Barbosa-Filho, J.M.; Patrocínio, M.C.A.; Viana, G.S.B.; et al. The anxiolytic-like effect of 6-styryl-2-pyrone in mice involves GABAergic mechanism of action. *Metab. Brain Dis.* **2018**, *33*, 139–149. [CrossRef] [PubMed]
64. Islam, M.T.; Riaz, T.A.; Ayatollahi, S.A.; Sharifi-Rad, J. Anxiolytic-like effect of *Urena lobata* (L.) in Swiss albino mice. *Clin. Phytoscience* **2021**, *7*, 11. [CrossRef]
65. Islam, M.T.; Martins, N.; Imran, M.; Hameed, A.; Ali, S.W.; Salehi, B.; Ahmad, I.; Hussain, A.; Sharifi-Rad, J. Anxiolytic-like effects of Moringa oleifera in Swiss mice. *Cell Mol Biol* **2020**, *66*, 73–77. [CrossRef]
66. Jeon, S.J.; Park, H.J.; Gao, Q.; Lee, H.E.; Park, S.J.; Hong, E.; Jang, D.S.; Shin, C.Y.; Cheong, J.H.; Ryu, J.H. Positive effects of β-amyrin on pentobarbital-induced sleep in mice via GABAergic neurotransmitter system. *Behav. Brain Res.* **2015**, *291*, 232–236. [CrossRef] [PubMed]
67. Liu, W.L.; Wu, B.F.; Shang, J.H.; Zhao, Y.L.; Huang, A.X. Moringa oleifera Lam Seed Oil Augments Pentobarbital-Induced Sleeping Behaviors in Mice via GABAergic Systems. *J. Agric. Food Chem.* **2020**, *68*, 3149–3162. [CrossRef] [PubMed]
68. Kim, R.E.; Mabunga, D.F.; Kim, H.J.; Han, S.H.; Kim, H.Y.; Shin, C.Y.; Kwon, K.J. Novel Therapeutics for Treating Sleep Disorders: New Perspectives on Maydis stigma. *Int. J. Mol. Sci.* **2022**, *23*, 4612. [CrossRef]
69. Akyuz, E.; Paudel, Y.N.; Polat, A.K.; Dundar, H.E.; Angelopoulou, E. Enlightening the neuroprotective effect of quercetin in epilepsy: From mechanism to therapeutic opportunities. *Epilepsy Behav.* **2021**, *115*, 107701. [CrossRef]
70. Cárdenas-Rodríguez, N.; González-Trujano, M.E.; Aguirre-Hernández, E.; Ruíz-García, M.; Sampieri, A., 3rd; Coballase-Urrutia, E.; Carmona-Aparicio, L. Anticonvulsant and antioxidant effects of Tilia americana var. mexicana and flavonoids constituents in the pentylenetetrazole-induced seizures. *Oxid. Med. Cell Longev.* **2014**, *2014*, 329172. [CrossRef]
71. Nieoczym, D.; Socała, K.; Raszewski, G.; Wlaź, P. Effect of quercetin and rutin in some acute seizure models in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2014**, *54*, 50–58. [CrossRef]
72. Sumbul, O.; Aygun, H. Chronic effects of different quercetin doses in penicillin-induced focal seizure model. *Neurosci. Lett.* **2021**, *753*, 135848. [CrossRef]
73. Tavakoli, Z.; Tahmasebi Dehkordi, H.; Lorigooini, Z.; Rahimi-Madiseh, M.; Korani, M.S.; Amini-Khoei, H. Anticonvulsant effect of quercetin in pentylenetetrazole (PTZ)-induced seizures in male mice: The role of anti-neuroinflammatory and anti-oxidative stress. *Int. Immunopharmacol.* **2023**, *116*, 109772. [CrossRef] [PubMed]
74. Bang, M.H.; Choi, S.Y.; Jang, T.O.; Kim, S.K.; Kwon, O.S.; Kang, T.C.; Won, M.H.; Park, J.; Baek, N.I. Phytol, SSADH inhibitory diterpenoid of Lactuca sativa. *Arch. Pharm. Res.* **2002**, *25*, 643–646. [CrossRef] [PubMed]
75. Aragão, G.F.; Carneiro, L.M.; Rota-Junior, A.P.; Bandeira, P.N.; de Lemos, T.L.; Viana, G.S. Alterations in brain amino acid metabolism and inhibitory effects on PKC are possibly correlated with anticonvulsant effects of the isomeric mixture of α- and β-amyrin from Protium heptaphyllum. *Pharm. Biol.* **2015**, *53*, 407–413. [CrossRef] [PubMed]
76. Singh, A.; Kukreti, R.; Saso, L.; Kukreti, S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. *Molecules* **2019**, *24*, 1583. [CrossRef] [PubMed]

77. Santos, C.C.d.M.P.; Salvadori, M.S.; Mota, V.G.; Costa, L.M.; de Almeida, A.A.C.; de Oliveira, G.A.L.; Costa, J.P.; de Sousa, D.P.; de Freitas, R.M.; de Almeida, R.N. Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. *Neurosci. J.* **2013**, *2013*, 949452. [CrossRef] [PubMed]
78. Costa, J.P.; Costa, D.A.; Almeida, R.N.; Freitas, R.M.; Sousa, D.P.; Fortes, A.C.; Patrício, C.C.M.; Soares, M.F.L.R. Applications of Phytol in Pharmaceutical Formulations Anxiolytic and Antidepressant Properties. *Chem. Biol. Interact.* **2011**, *240*, 63–70.
79. Park, S.J.; Ahn, Y.J.; Oh, S.R.; Lee, Y.; Kwon, G.; Woo, H.; Lee, H.E.; Jang, D.S.; Jung, J.W.; Ryu, J.H. Amyrin attenuates scopolamine-induced cognitive impairment in mice. *Biol. Pharm. Bull.* **2014**, *37*, 1207–1213. [CrossRef]
80. Simão da Silva, K.A.B.; Paszcuk, A.F.; Passos, G.F.; Silva, E.S.; Bento, A.F.; Meotti, F.C.; Calixto, J.B. Activation of cannabinoid receptors by the pentacyclic triterpene α,β-amyrin inhibits inflammatory and neuropathic persistent pain in mice. *Pain* **2011**, *152*, 1872–1887. [CrossRef]
81. Park, H.J.; Kwon, H.; Lee, J.H.; Cho, E.; Lee, Y.C.; Moon, M.; Jun, M.; Kim, D.H.; Jung, J.W. β-Amyrin Ameliorates Alzheimer's Disease-Like Aberrant Synaptic Plasticity in the Mouse Hippocampus. *Biomol. Ther.* **2020**, *28*, 74–82. [CrossRef]
82. Lee, A.; Tariq, A.; Lau, G.; Tok, N.W.K.; Tam, W.W.S.; Ho, C.S.H. Vitamin E, Alpha-Tocopherol, and Its Effects on Depression and Anxiety: A Systematic Review and Meta-Analysis. *Nutrients* **2022**, *14*, 656. [CrossRef]
83. Vanmierlo, T.; Weingärtner, O.; van der Pol, S.; Husche, C.; Kerksiek, A.; Friedrichs, S.; Sijbrands, E.; Steinbusch, H.; Grimm, M.; Hartmann, T.; et al. Dietary intake of plant sterols stably increases plant sterol levels in the murine brain. *J. Lipid Res.* **2012**, *53*, 726–735. [CrossRef]
84. Sharma, N.; Tan, M.A.; An, S.S.A. Phytosterols: Potential Metabolic Modulators in Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2021**, *22*, 12255. [CrossRef] [PubMed]
85. Griffiths, W.J.; Wang, Y. Sterolomics in biology, biochemistry, medicine. *Trends Anal. Chem.* **2019**, *120*, 115280. [CrossRef] [PubMed]
86. Panayotis, N.; Freund, P.A.; Marvaldi, L.; Shalit, T.; Brandis, A.; Mehlman, T.; Tsoory, M.M.; Fainzilber, M. β-sitosterol reduces anxiety and synergizes with established anxiolytic drugs in mice. *Cell Rep. Med.* **2021**, *2*, 100281. [CrossRef] [PubMed]
87. Maldonado-Peralta, M.A.; De Los Santos, G.G.; García-Nava, J.R.; Ramírez-Herrera, C.; Hernández-Livera, A.; Valdez-Carrazco, J.M.; Corona-Torres, T.; Cetina-Alcalá, V.M. Seed viability and vigour of two nanche species (*Malpighia mexicana* and *Byrsonima crassifolia*). *Seed Sci. Technol.* **2016**, *44*, 168–176. [CrossRef]
88. Maldonado-Peralta, M.A.; García De Los Santos, G.; García-Nava, J.R.; Rojas-García, A.R. Propagación vegetativa de nanche *Malpighia mexicana* y *Byrsonima crassifolia*. *Rev. Mex. Cienc. Agrícolas* **2017**, *8*, 611–619. [CrossRef]