

# EFFECTS OF THE ETHANOLIC EXTRACT OF SCHINUS TEREBINTHIFOLIUS RADDI FRUITS ON LOCOMOTOR ACTIVITY AND ANXIETY-LIKE BEHAVIORS IN WISTAR RATS

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DOI: [10.5281/zenodo.12289228](https://doi.org/10.5281/zenodo.12289228)

## Abstract

*Schinus terebinthifolius Raddi*, a member of the Anacardiaceae family, is a perennial shrub commonly known as the Brazilian pepper tree. The fruits of this plant have garnered significant attention due to their aromatic flavor and potential medicinal properties, which may contribute to the prevention and treatment of various diseases. This study aimed to investigate the effects of a single oral administration of various doses of the ethanolic extract of *S. terebinthifolius* fruits on behavioral outcomes in the open field and elevated plus maze tests. The extract was orally administered via gavage at doses of 2.5, 5, 25, 50, and 250 mg/kg (n = 5/group). The open field and elevated plus maze tests were used to assess locomotor and anxiety-like behaviors 30 minutes post-administration of the extract. The behavioral evaluation revealed that at a dose of 50mg/kg, the extract significantly increased the number of total squares visited in the open field test. Additionally, all doses used in the study increased statistically the time spent in the open arms of the elevated plus maze test. Thus, the ethanolic extract of *S. terebinthifolius* fruits may have anxiolytic effects.

**Keywords:** *Schinus Terebinthifolius Raddi*, Locomotor Activity, Anxiety, Wistar Rats.

## 1. INTRODUCTION

*Schinus terebinthifolius Raddi*, commonly known as the Brazilian pepper tree or pink pepper, is a plant species native to South/and Central America, and Africa [1]. Extracts derived from *Schinus terebinthifolius* have a wide range of beneficial pharmacological properties, such as antioxidant, anti-inflammatory, antiproliferative, and antibacterial properties [2]. Anxiety disorders are a group of mental health conditions characterized by excessive fear, worry, and apprehension [3–5]. These disorders can significantly impact an individual's quality of life [6–8] and are often treated with pharmacological interventions, such as benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) [9,10]. However, these medications can have adverse side effects and may not be effective for everyone [11]. Therefore, there is a need for the development of alternative treatments with fewer side effects and improved efficacy.

The use of plant-based medicines has gained increasing attention as a potential alternative to conventional pharmacological treatments for several disorders including anxiety [12–22]. Several studies have investigated the anxiolytic effects of various plant extracts in animal models, including *Passiflora incarnata* [23], *Valeriana officinalis* [24], and *Melissa officinalis* [25]. These studies have provided promising results, suggesting that plant-based medicines may be a viable option for the treatment of anxiety disorders. The present study was undertaken to investigate the

effects of a single oral administration of various doses of the ethanolic extract of *S. terebinthifolius* fruits on locomotor activity and anxiety-like behaviors in Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant materials and the preparation of *Schinus terebinthifolius* extract

The berries of *Schinus terebinthifolius* Raddi were collected from Kenitra city, which is situated in Morocco. The species was identified, and a sample was preserved in the herbarium of the Biology and Health laboratory at the Faculty of Science, Ibn Tofail University, Kenitra, Morocco. The chosen berries were subjected to a drying process in an oven at 30 °C for a night and then ground, the obtained powder was extracted first with n-hexane in a Soxhlet apparatus. The suspension was then filtered using filter paper. The n-hexane was subsequently recycled at 55°C by the utilization of a vacuum rotary evaporator [26]. The defatted plant material was left in an oven at a temperature of 25°C overnight, then a second extraction with ethanol was carried out using the same apparatus (soxhlet) to recover extracts.

### 2.2 Animals

Thirty adults female Wistar rats (weighing 214, 56±0,36g), aged 3 months old, were obtained from the breeding center of the faculty of science, Ibn Tofail University. The rats were housed in specific cages under regulated circumstances, with a constant temperature of 22 ± 2°C and a 12-hour light/12-hour dark cycle (with lights on at 6 am). They were provided with free access to food and water (standard diet). All experimental procedures followed the guidelines outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Every attempt was made to minimize animal suffering and reduce the number of animals used in the study.

### 2.3 Experimental design

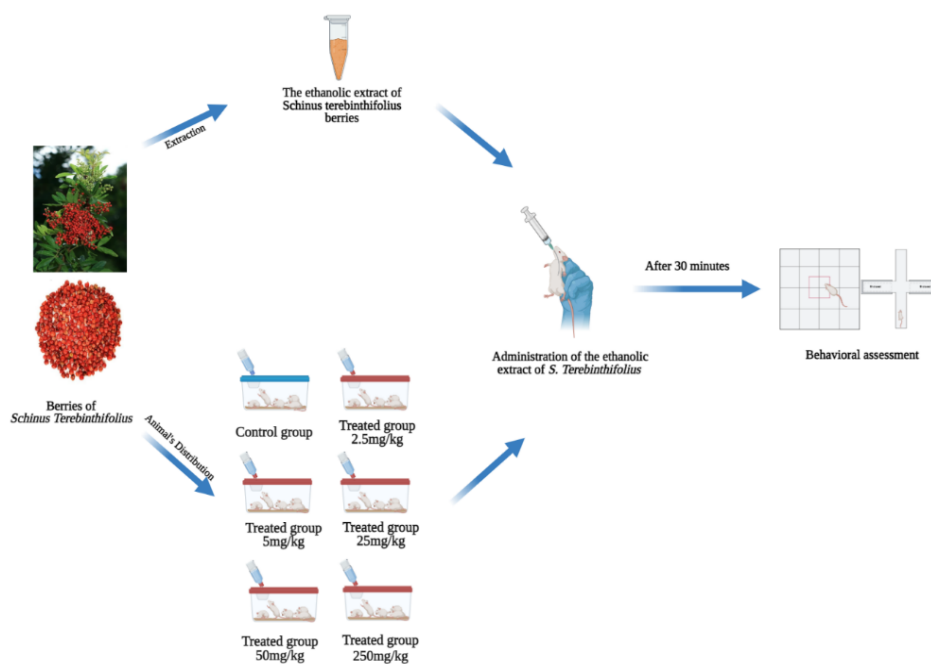


Figure 1: Study Design

The study was carried out on 30 female Wistar rats. They were divided into 6 groups (n=6). The control group received distilled water orally at 1ml/100g of body weight, and the treated groups with the ethanolic extract received various doses of the extract as follows: 2.5, 5, 25, 50, and 250mg/kg.

## **2.4 Behavioral assessment**

The open field (OF) and the elevated plus maze (EPM) Tests were used to investigate the general locomotor activity and anxious-like behavior in rats

### **2.4.1 The open-field test**

The OF test was used to assess general locomotor activity and to measure anxiety. The assessment took place in a white enclosure of dimensions: 100x100x40cm; whose floor is divided into 25 equal squares (16 peripherals and 9 central) the slide was illuminated by a 60W lamp and controlled by a computer-linked camera [27].

During the test, each rat was placed in the center for 7 minutes, and the number of total squares visited was measured to assess the general locomotor activity. Additionally, the number of returns into the central zone as well as the time spent in this area was measured to assess anxiety. In between testing, the apparatus was cleaned using ethanol. The central area of a new environment is inherently anxiogenic for rodents, meaning that anxious animals tend to avoid the center of the OF test [28].

### **2.4.2 The Elevated Plus Maze test**

The EPM test is widely used to assess anxiety-like behavior in rodents. The apparatus consists of a "+" shaped maze elevated from the ground (50cm) with two open arms (50x10cm) and two closed arms (50x10x40cm), connected by a platform (10x10cm). A 60 W lamp illuminated the central platform, giving the rat an aversive spatial condition. Each animal was positioned on the platform facing an open arm and its activity was recorded for 5 minutes. In between testing, the apparatus was cleaned using ethanol. The duration spent in each arm and the frequency of entries in the closed and open arms were noted from a video clip. Rat level anxiety was determined by the time spent in the open arms and the number of entries into these arms [29–31].

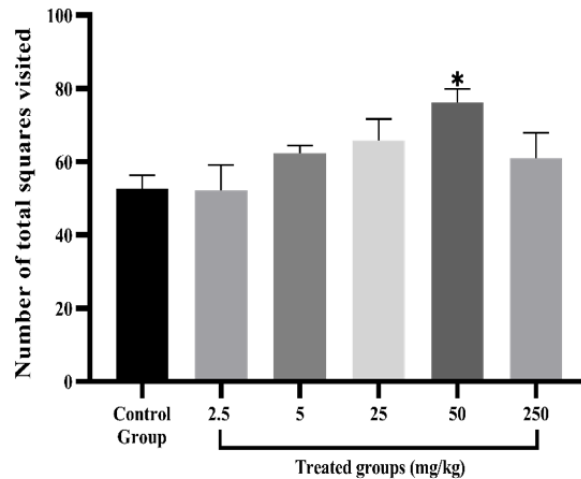
## **2.5 Statistical Analysis**

Values were presented as mean  $\pm$  standard error. a one-way ANOVA analysis made it possible to evaluate the effect of the plant separately. When there was a significant interaction ( $p < 0.05$ ), we performed a complementary statistical test, the Tukey Post-Hoc Test. This method allows significant differences to be observed between different pairs of data. Statistical analyzes were carried out using GraphPad Prism 7 software...

## **3. RESULTS**

### **3.1 General locomotor activity in the open field test**

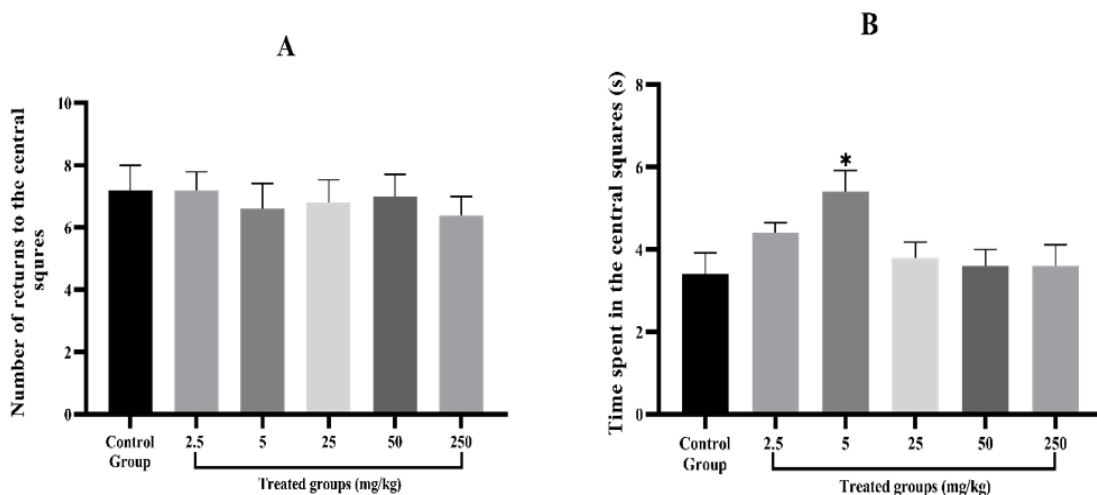
Following the administration of the ethanolic extract of *S. terebinthifolius* fruits, treated groups with 5, 25, 50, and 250mg/kg of the extract, exhibited an increase in the number of total squares visited compared to the control group. Notably, the group administered the dose of 50mg/kg of the extract demonstrated a statistically significant increase in the number of squares visited ( $F_{5, 24} = 2.976$ ,  $P = 0.0314$ ) (Figure 2).



**Figure 2: Rats' behavioral performance in the open field test. The number of total squares visited 30 minutes post administration of the ethanolic extract of *Schinus terebinthifolius*, measured in seconds. The results are presented as Mean ±SEM. The level of significance is 0.05 (One Way ANOVA and post hoc multiple comparisons)**

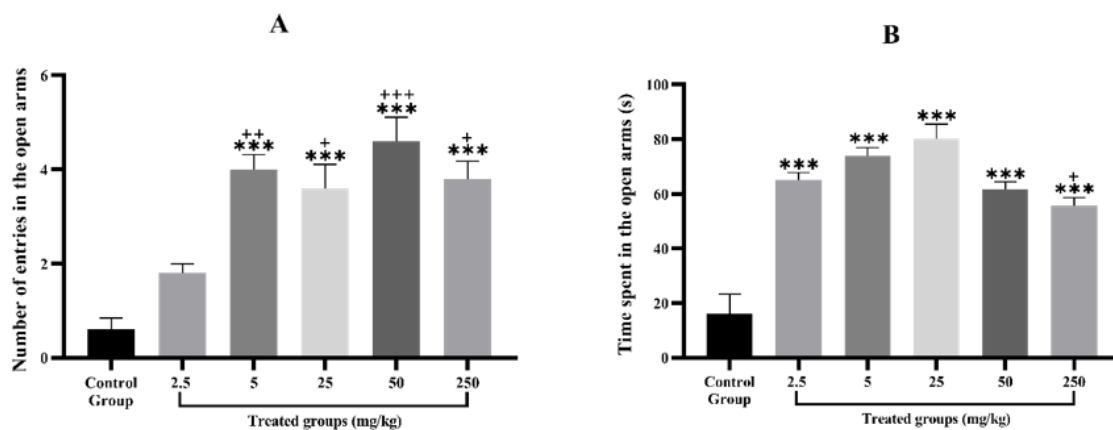
### 3.2 Anxiety assay

Two tests were used to evaluate anxiety-like behavior, the OF test (Fig.3) and the EPM test (Fig.4). In the OF test (Fig.3), the extract did not induce a significant change in the number of returns to the central squares between the treated groups and the control group ( $F_{5, 24} = 0.2105, P=0.9547$ ). However, all treated groups displayed an elevation in the time spent in the central area compared to the control group. Notably, the group that administered the dose of 5mg/kg of the extract showed a statistically significant increase in the time spent in the central area compared to the control group ( $F_{5, 24} = 2.982, P=0.0312$ ).



**Figure 3: Rats' behavioral performance in the open field test. A. The number of returns to the central squares & B. The time spent the central squares 30 minutes post administration of the ethanolic extract of *Schinus terebinthifolius*. The results are presented as Mean ±SEM. The level of significance is 0.05. \*p < 0.05 (One Way ANOVA and post hoc multiple comparisons)**

In the EPM test (Fig.3), the results show that treated rats at doses of 5, 25, 50, and 250mg/kg displayed a highly significant elevation in the number of entries in the open arms of the test compared to control rats ( $F_{5, 24} = 16.37, P < 0.0001$ ). Furthermore, these doses showed a marked increase in the number of entries in the open arms compared to treated rats with 2.5mg/kg of the same extract. Additionally, all treated groups showed an increase in the time spent in the open arms of the test compared to the control group ( $F_{5, 24} = 27.33, P < 0.0001$ ). Notably, treated rats at a dose of 250mg/kg displayed a significant decrease in the time spent in the open arms compared to the group treated with 25mg/kg of the extract.



**Figure 4: Rats' behavioral performance in the elevated plus maze test A. The number of entries in the open arms and B. The time spent in the open arms 30 minutes post administration of ethanolic extract of *Schinus terebinthifolius*. The results are presented as Mean  $\pm$ SEM. The level of significance is 0.05. \*\*\*p < 0.001 versus the control group, +p < 0.05, ++p < 0.01, +++p < 0.001 versus the group of 2.5 mg/kg (Fig.3A) and the group of 25mg/kg (Fig. 3B) (One Way ANOVA and post hoc multiple comparisons)**

#### 4. DISCUSSION

The present study aimed to evaluate the effects of a single oral administration of varying doses of the ethanolic extract derived from *S. terebinthifolius* fruits on the behavior of rats. The doses tested ranged from 2.5 to 250mg/kg. Two tests were used to assess the behavioral outcomes the open field test and the elevated plus maze test. Our results revealed that at a dose of 50mg/kg, the rats significantly increased the number of total squares visited in the OF test, suggesting that the extract may have a stimulatory effect on locomotor activity, potentially due to its antioxidant and anti-inflammatory properties. This finding is consistent with previous studies that have demonstrated the stimulatory impact of *S. terebinthifolius* extracts on locomotor activity, Scheid et al. [32] found that the extract from leaves of *S. terebinthifolius* increased locomotor activity in the OF test. Similarly, Sereniki et al. reported that the extract from the stem bark of *S. terebinthifolius* enhanced locomotor activity in the rotarod test. Additionally, de Lima [33] observed that the extract from fruits of *S. terebinthifolius* increased locomotor activity in the OF test. When evaluating anxiety-like behavior in the OF and the EPM test.

Our results showed that all treated groups spent more time in the open field's central squares than the control group, particularly at the dose of 5mg/kg. Similarly, these groups spent more time in the open arms of the EPM test than the control group. This suggests that the ethanolic extract of *Schinus terebinthifolius* may have anxiolytic properties, as increased time spent in the central squares of the OF test and in the open arms of the EPM are often associated with reduced anxiety. Our results are consistent with a study conducted on mice by Lima et al. [34], which evaluated the anxiolytic activity of *S. terebinthifolius*. Other studies have shown that relation between anxiety or depression and some diseases are related to a mental illness like depression and anxiety [34-40] and the ability of plant or vitamin supplementation to modulate the physiology and the behavior of an individual [41-44].

This study found that the extract from *S. terebinthifolius* leaves significantly increased the time spent in the open arms while reducing the time spent in the closed arms, suggesting that the extract may modulate monoaminergic neurotransmission.

Monoaminergic neurotransmission is known to play a crucial role in regulating mood and anxiety-related behaviors, and the modulation of these neurotransmitter systems could be the mechanism by which *Schinus terebinthifolius* exerts its anxiolytic effects in mouse models of anxiety [45]. The potential therapeutic of phytochemical compounds that this plant contained can be explored in the management of some diseases [46-49] as can be used negatively as psychoactive substances [50]

## 5. CONCLUSION

In conclusion, this study shows that ethanolic extract of *S. terebinthifolius* fruits may have anxiolytic effects and used as a drug in the treatment of anxiety.

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