Latin American Journal of Pharmacy
(Formerly *Acta Farmacéutica Bonaerense*)

Lat. Am. J. Pharm. 2023; 42(6): 34-42

Phytochemical and Pharmacological Evaluation of *Cucumis melo* Var. momordica (Roxb.) Linn for anti-anxiety activity.

Priya Rai[1], Pallavi Tiwari*[1], Dr. Manoj Kumar Mishra[1], Mayank Srivastava[2], Saurav Ghoshal[1]

Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj, Uttar Pradesh, India
Mahrishi College of Pharmacy, Bharwari, Kaushambi, Uttar Pradesh, India

***Corresponding author**:

Dr. Pallavi Tiwari

E mail: pallavitiwari25@gmail.com Contact No.: +91 9721683816

Abstract:

This research aimed to explore the phytochemical composition and pharmacological potential of Cucumis melo Var. Momordica (Roxb.) Linn as a natural remedy for anxiety disorders. We employed advanced analytical techniques, including chromatography and spectroscopy, to identify and quantify the phytochemical constituents of Cucumis melo Var. Momordica. The anti-anxiety activity was assessed using established behavioral paradigms and biochemical assays in animal models. Neurobiological mechanisms were elucidated through neurotransmitter profiling and neural circuitry analysis. Safety and tolerability were evaluated to determine its suitability for therapeutic use. Our findings revealed a diverse phytochemical profile in Cucumis melo Var. momordica, including triterpenoids, flavonoids, alkaloids, and polypeptides. The plant extract demonstrated significant anti-anxiety effects, as evidenced by reduced anxiety-related behaviors in animal models. Neurobiological investigations suggested modulation of neurotransmitter systems and neural pathways associated with anxiety regulation. Importantly, the plant extract exhibited a favorable safety profile with minimal adverse effects. Cucumis melo Var. Momordica shows promise as a natural anxiolytic agent, with its phytochemical constituents exerting anti-anxiety effects through modulation of neurobiological pathways. These findings provide valuable insights into the potential therapeutic use of bitter melon in managing anxiety disorders and offer a foundation for further research and development of botanical interventions for anxiety management.

Keywords: Cucumis melo Var. momordica, Bitter melon, Phytochemicals, Pharmacological evaluation, Anti-anxiety activity, anxiety disorders

1. Introduction

Anxiety disorders represent a significant global health concern, affecting millions of individuals across diverse age groups and populations [1]. Characterized by excessive worry, fear, and nervousness, these conditions can have a debilitating impact on one's quality of life, leading to impairments in daily functioning, reduced productivity, and profound distress [2]. The multifaceted nature of anxiety disorders, which encompass generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, and specific phobias, necessitates a holistic approach to their management and treatment [3]. While conventional pharmaceutical interventions such as benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) have demonstrated efficacy in managing anxiety symptoms, they often come with undesirable side effects, including sedation, addiction potential, and withdrawal symptoms [4].

Consequently, there is a growing interest in identifying natural remedies and complementary therapies with anti-anxiety properties, offering a safer and more sustainable approach to anxiety management [5]. Plants have been a cornerstone of traditional medicine systems worldwide, providing a rich source of bioactive compounds that exhibit therapeutic potential against a wide array of ailments, including anxiety disorders.

Among these, *Cucumis melo Var. momordica (Roxb.) Linn*, commonly known as bitter melon or karela, has emerged as a plant of interest due to its historical use in traditional medicine and its diverse phytochemical composition. *Cucumis melo Var. momordica*, a member of the Cucurbitaceae family, is a widely cultivated plant primarily found in tropical and subtropical regions [6,7]. It is renowned for its distinctive bitter taste and has been a dietary staple in many cuisines, particularly in Asia, where it is valued for its culinary and medicinal properties. The plant is characterized by its vine-like growth, lobed leaves, and distinctive oblong, warty fruits that turn from green to yellow or orange when ripe [8]. In traditional medicine systems such as Ayurveda, Traditional Chinese Medicine (TCM), and folk remedies in various cultures, *Cucumis melo Var. momordica* has been used to treat a range of ailments, including diabetes, gastrointestinal disorders, and infections. Its potential as an anxiolytic agent, however, has only recently gained attention in the scientific community.The therapeutic properties of *Cucumis meloVar. momordica* are attributed to its rich phytochemical composition, which includes a variety of bioactive compounds [9,10].

Preliminary investigations have identified several classes of phytochemicals present in this plant, including, triterpenoidssuch as momordicin, charantin, and cucurbitacin, which have exhibited anti-inflammatory and antioxidant properties in various studies.Flavonoids like quercetin and kaempferol have been found in *Cucumis meloVar. momordica* and are known for their neuroprotective effects and potential in managing mood disorders. Certain alkaloids, though in lesser quantities, have also been detected in bitter melon and may contribute to its pharmacological activity. Bioactive polypeptides found in the plant have shown promise in regulating neurotransmitters and modulating the central nervous system.These phytochemicals are believed to work synergistically to produce the observed medicinal effects of *Cucumis melo Var. momordica*.Given the growing demand for alternative treatments for anxiety disorders and the rich phytochemical composition of *Cucumis melo Var. momordica*, it is imperative to explore its potential as a natural anxiolytic agent [11,12].

Scientific investigations into the anti-anxiety properties of this plant are not only intriguing but also hold promise for the development of novel therapies with fewer adverse effects.This research aims to comprehensively evaluate the phytochemical constituents of *Cucumis melo Var. momordica* and their pharmacological effects on anxiety-related behavior and neurobiology [13].

1. MATERIAL AND METHOD

1.1. Plant Material

The seeds of (CMMF) were collected from the river side area of district Sultanpur, UP, India in the month of August to September. And it authenticated from Botanical Survey of India, Prayagraj by taxonomist voucher specimen No. 1206230014388. Collected seed air dried under shade & moisture was evaporated of the flower and then powdered it.

1.2. Extract preparation

3kg seed of *Cucumis melo var. momordica* (Roxb). were firstly air dried then it was dried in tray dryer under the controlled condition and got converted into powder form,it then 300gm dried seed powder macerated with 50% ethanol by maceration process. And ethanolic extract was placed at room temperature for seven days with continuous stirring after seven days ethanolic extract was filtered. Alcoholic mass evaporated with Soxhlet apparatus at (50-60^{0}C) for18 hrs some alcoholic extracts obtained. And it was placed in water bath (45^{0}C) some time toproduce a semisolid massof *Cucumis melo Var. momordica*(Roxb). and stored in airtightcontainer [14-16].

1.3. PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was conducted to identify the presence of specific classes of phytochemical compounds in the extracts obtained from Cucumis melo Var. momordica (Roxb.) Linn. The screening involved a series of qualitative chemical tests, each targeting different groups of phytochemicals. The following tests were performed i.e. Alkaloids Test, Flavonoids Test, Tannins Test, Saponins Test, Terpenoids Test, Phenols Test, Glycosides Test, Proteins and Amino Acids Test, Carbohydrates Test, Steroids and Triterpenoids Test, Phytosterols Test etc [17,18].

2.4 EXPERIMENTAL ANIMALS

Young wistar albino rats (male) weight 45-65 gm obtained from central drug research institute animal house Lucknow. Polypropylene cages used for storage of animal under standard conditions, where 12 hr dark light cycle maintain at 25 ± 2ºC. Standard pellet diet and water ad libitum was given to animal during or before the experiment. Animals was placed to quarantine area for acclimatized to animal house condition for at least 2 weak before the experiment. IAEC has approved the experimental protocol IAEC/010/03/23.

2.5 DETERMINATIONOFORAL ACUTE TOXICITYSTUDY (LD_{50})

LD_{50} dose determination was carried out in rats by OECD Guidelines 423. One dose of the drugextract (10, 100, 500, or 1000 mg/kg) in the app. quantity of H_2O was given orally by gavage to various groups of rats (six rats in every group) Continuous access of H_2O and food were given to the animals were deprived of food for two hrs before dosing and four hrs after drug administration. For the first twelve hrs, the animals were initially monitored continuously for any adverse effects for four hrs and then monitored at one hrs intervals. They were then monitored two times a day for any abnormal changes in the study period (which lasted 14 days) as reported by [19,20]. The (LD_{50}) dose which comprises fifty percent of ethanolic extract of *Cucumis melo Var. momordica* (Roxb). Seed was 2000 mg/kg. 1/10 of the max. dose of CMMF was subjected toxicity for pharmacological study (i.e., 250 mg/kg and its two times strength of 500mg/kgof bodywt.).

2.6 EVALUATION MODELS FOR ANXIOLYTIC ACTIVITY OF *CUCUMIS MELO VAR. MOMORDICA*(ROXB) SEED

ELEVATED PLUS –MAZES (EPM)

EPM has two ars i.e open (35 X 6cm) and closed arms (35 X 6 X 15 cm). The EPM was raised to a height of 50 cm in order to assess the animal's degree of anxiousness. Transfer Latency and Memory Retention are the two main parameters employed in EPM. In Transfer Latency, the animal is placed on the edge of the open arm, while the mouse is placed far away from the platform's middle. The amount of time it takes the rat to enter one of its closed appendices with all four legs is recorded as "Transfer latency". If rats did not enter the closed arm after three minutes, they were carefully pushed into one of the locked arms within 180 seconds, and in this instance, the time limit was set at 180 seconds. The mouse was given permission to explore the labyrinth for the next 15 seconds before being put back in its cage of origin. On the following day, 24 hours after the primary day's preliminary, memory maintenance is examined. The labyrinth was meticulously cleaned with 30% ethanol before each meeting to remove any odours [21,22].

Albino mice of either sex weighing between 45-65 g were divided into 5 groups of 6 mice in each were fasted overnight prior to the test but water was supplied ad libitum.

Group1: Positive control group,which received saline solution use as a vehicle.

Group2: Diazepam (1mg/kg, p.o.).

Group3: Chemically induced Anxiety rat, which was cured with CMMS 250 mg/kg body wt.

Group4: Chemically induced Anxiety rat, which was cured with CMMS 500 mg/kg body wt.

Group 5: Chemically induced Anxiety rat, which were cured with the std. drug Diazepam at 10mg/kg as per body wt.

2.7 LIGHT-DARK TEST(LDT)

The Light-Dark Test is a widely used behavioral test in preclinical research for assessing anxiety-like behavior in rodents, including mice. This test takes advantage of the natural aversion of rodents to brightly lit, open spaces and their preference for dark, enclosed areas. A specially designed testing chamber is used, consisting of two compartments, one dark and one brightly illuminated, connected by an opening or tunnel. The dark compartment is typically black or covered with opaque material to create a dark, enclosed space.

The light compartment is well-lit with white light or other sources of illumination. Prior to testing, mice are allowed to habituate to the testing room for a suitable period to reduce stress and novelty-induced behavior. The mouse is placed in the center of the dark compartment of the apparatus. A partition (often removable) is placed at the entrance of the tunnel, preventing access to the light compartment. At the beginning of the test, the partition is removed, allowing the mouse to choose between the light and dark compartments. The test duration can vary but typically lasts for 5 to 10 minutes. An observer records the following behaviors:

Latency to enter the light compartment (the time taken for the mouse to cross into the light compartment for the first time). Total time spent in the light compartment. Total number of transitions between compartments (crossings). Time spent in the dark compartment (a measure of anxiety-related behavior, as longer durations in the dark suggest higher anxiety levels). The test is usually terminated after a predetermined duration or when the mouse remains in one compartment for a set period (e.g., 300 seconds). Data collected during the test can be analyzed to assess anxiety-like behavior. Parameters such as increased latency to enter the light compartment, decreased time spent in the light compartment, and reduced number of transitions indicate higher levels of anxiety-like behavior.

Between tests, the apparatus is thoroughly cleaned and sterilized to eliminate any residual odors and potential biases. The Light-Dark Test provides valuable insights into the anxiety-related behaviors of mice and is often used alongside other behavioral tests to comprehensively assess anxiety. It is important to note that the results of the test should be interpreted in conjunction with findings from other anxiety-related assays and in consideration of individual variations in behavior. This test is a useful tool for studying the effects of plant extracts, pharmaceuticals, or other interventions on anxiety-like behaviors in animal models [23-25].

Albino mice of either sex weighing between 45-65 g were divided into 5 groups of 6 mice in each were fasted overnight prior to the test but water was supplied ad libitum.

Group1: Positive control group,which received saline solution use as a vehicle.

Group2: Diazepam (1mg/kg, p.o.).

Group3: Chemically induced Anxiety rat, which was cured with CMMS 250 mg/kg body wt.

Group4: Chemically induced Anxiety rat, which was cured with CMMS 500 mg/kg body wt.

Group 5: Chemically induced Anxiety rat, which were cured with the std. drug Diazepam at 10mg/kg as per body wt.

The treatment was given once daily for 7^{th} days. On 7^{th} day 60min after administration of the vehicle, standard drug and test extract to different groups, each mouse was placed in the light chamber facing the opening into the dark chamber, and the following observations were recorded manually during a 5-min trial: Time spent in the light compartment, number of squares crossed and duration of immobility.

2.8 STATISTICAL ANALYSIS

The data obtained from the above findings were subjected analysis following one-way ANOVA followed by Tukey's Kramer Comparison Test to assess the statistical significance of the results using Graph Pad Prism 8.0 software's. p-values less than 0.05 were considered as statistically significant.

3 RESULTS

3.1 PHYTOCHEMICALANALYSIS

The result of phytochemical analysis of crude powder of seed of *Cucumis melo Var. Momordica* (Roxb.*)* powder extract shown in Table.

Table 1: Phytochemical Analysis of crude powder of seed of *Cucumis melo Var. Momordica* (Roxb.) powder extract

S. NO.	Test Performed	Ethanolic extract	Aqueous extract
1.	Alkaloids	+	+
2.	Saponins	+	+
3	Steroid	+	+
4	Carbohydrates	+	+
5	Anthraquinone Glycosides	+	+
6	CardiacGlycosides	+	+
7	Tannins	+	+
8	Proteins	+	+
9	Flavonoids Test	++	+

+= Presence; = Absence

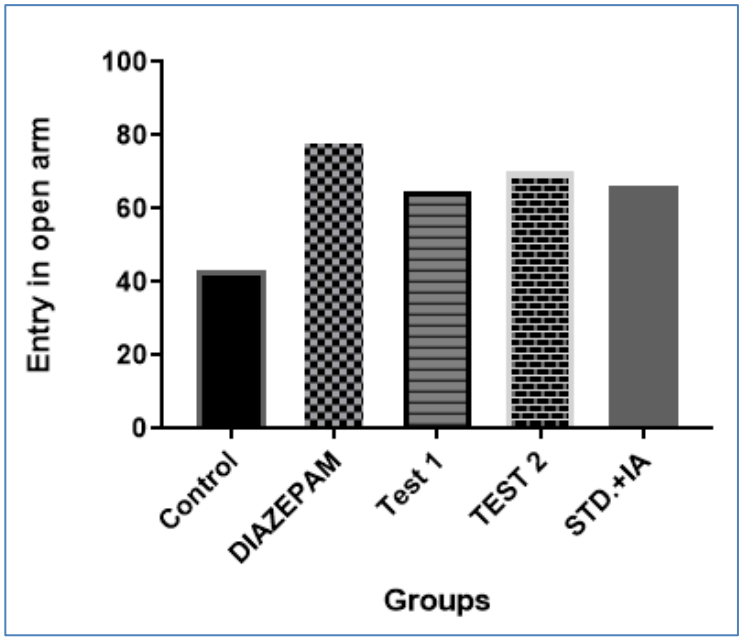
3.3 ANTI-ANXIETY ACTIVITY

The effect of oral administration of ethanolic extract of *Cucumis melo Var. Momordica* (Roxb). Seeds (250 mg/kg and 500 mg/kg of body weight) on anti-anxiety level of animals in 21 days is depicted in Table.

Table 3: Effects of ethanol extract of *Cucumis melo Var. momordica* (Roxb). Seed by EPM (CMMS) (Mean±SD)

S. NO.	Treatment groups	% Entry into open arm	% time spent into open arm
1	Control	43.48± 2.34	38.46±2.04
2	Std (Diazepam) 1mg/kg, p.o	79.30±5.44	75.78±4.74
3	Plant Extract 250mg/kg,p.o+m-cpp	64.65±3.26	62.25±2.36
4	Plant Extract 500mg/kg,p.o+ m-cpp	70.06±2.23	68.31±2.45
5	m-Cpp induced anxiety+ Diazepam	66.25±1.23	64.25±1.25

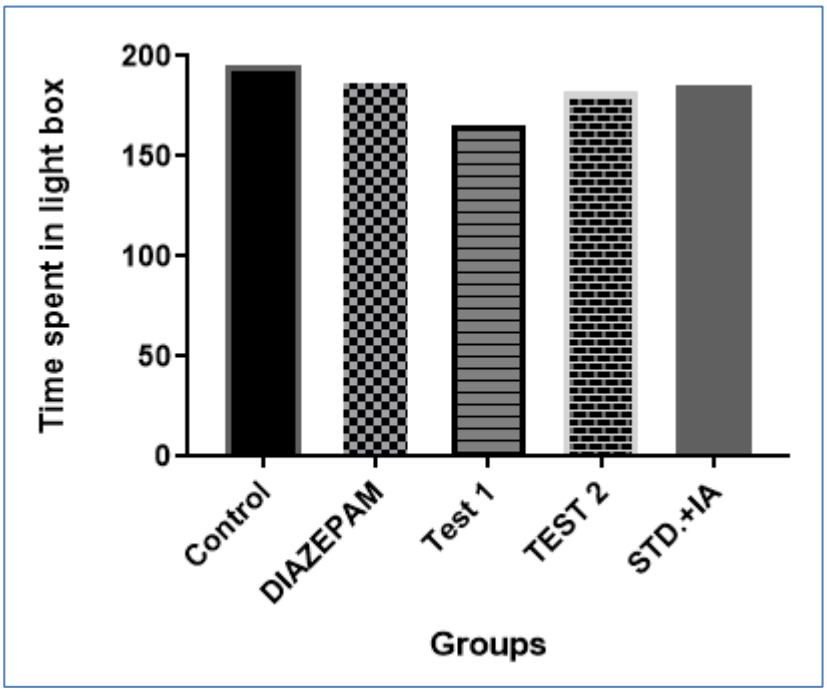
Values as Mean SEM (n=6), ns non-significant, *p<0.05, **p<0.001, ***p<0.001 as compared to control.



Graph showing parameters of rat behavior using Elevated Plus Maze

Table 4: Effects of ethanolic extract of *Cucumis melo Var. momordica* (Roxb). Seed by Light dark test (CMMS) (Mean±SD).

S. NO.	Treatment	Time Spent in Lighted Box	No. of square crossed	Duration of immobility
1	Control	95.23±3.52	11.25±2.64	36.23±3.31
2	Std (Diazepam) 1mg/kg, p.o	189.21±12.21	32.02± 2.45	17.65±1.05
3	Plant Extract 250mg/kg,p.o+m-cpp	165.21±11.23	25.64±1.18	23.25±2.33
4	Plant Extract 500mg/kg,p.o+m-cpp	182.26±9.33	28.36±2.18	24.25±1.33
5	m-Cpp induced anxiety+ Diazepam	185.36±7.33	27.33±3.14	24.25±1.05



Graph showing behavior of rat on light and dark test.

DISCUSSION

The present study was carried out to evaluate anti-anxiety activity of the *Cucumis melo Var. momordica* using animal models based on exploratory behavior: Elevated plus maze (EPM), and Light/dark transition test. It was found that *Cucumis melo Var. momordica seed* extract possess significant anti- anxiety activity in a dose dependent manner.

In the EPM test, Cucumis melo Var. momordica. extract was orally administered at doses 250mg/kg and 500mg/kg for 7 days. On the 8thday, 1hr after the administration it was observed from the study that it induced an anxiolytic-like effect in mice as it increased the number of open arm entries and the time spent on open arms respectively as compared to the control group of open arm entries and time spent in open arms.

In light/ dark transition test, the number of entries into the light chamber or transitions between light and dark chamber and the time spent in the light chamber are taken as anxiety indices. Mice was treated with *Cucumis melo Var. momordica*. extract for 7 days and on the 1 hour after the administration on the 8th day, it showed significant increase in the number of entries into the light chamber and the time spent in the light chamber as compared to the control group.

The increase in the parameters may be linked with the anxiolytic-like effect of the *Cucumis melo Var. momordica*.

In summary, the results of the present study demonstrated that the mice treated with the *Cucumis melo Var. momordica extract* produced significant anxiolytic- like effects in all the animal models of anxiety, when compared to the control group.

HISTOPATHOLOGY:

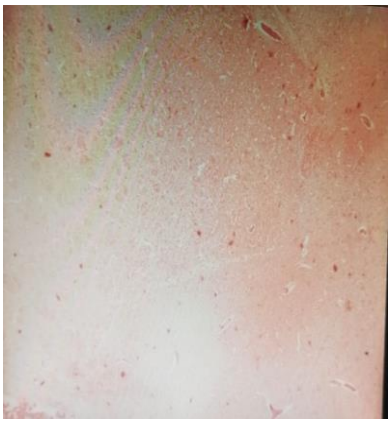


Fig. shows healthy neuron cells.

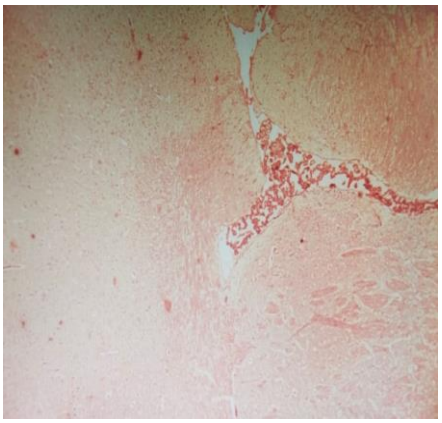


Fig. shows necrosis in neurotic cells

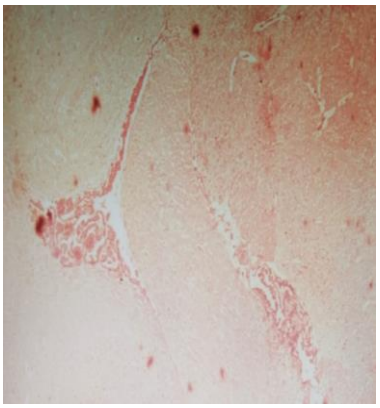


Fig. show regeneration of neurotic cells.

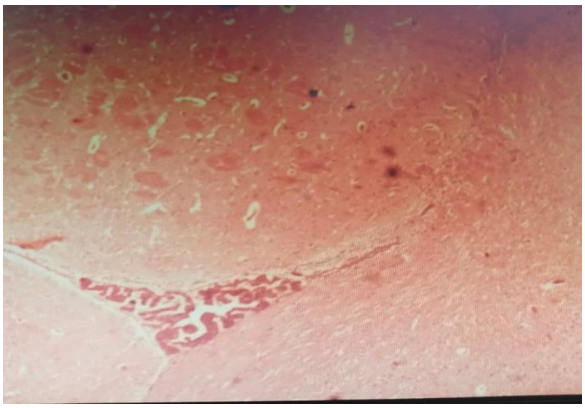


Fig. shows regeneration and recovery of neurotic cells.

4. CONCLUSION:

The present study was undertaken to study the antianxiety activity of ethanol extract of *Cucumis melo Var. momordica*. Acute oral toxicity studies revealed that the extract is safe till 2000mg/kg. For the evaluation of the antianxiety activity, elevated plus maze, and light dark transition was considered. Diazepam was taken as the reference standard and the extract was orally administered in 2 different doses of 250mg/kg (lower dose), and 500mg/kg (higher dose). The antianxiety activity of the plant was assessed in terms of various parameters utilized in the models considered for the present study. The parameters include no. of open arm entry, time spent in open arm, no. of entries to light side, time spent in light side.

The results obtained were compared with the normal group and standard group. The comparative analysis showed the significance of the ethanolic extract of *Cucumis melo Var. momordica*. in anti-anxiety ability. Histopathology study shows normal neurons and toxic groups shows necrotic cells.

Based on the results confirmed from the present study, it was concluded that the ethanolic extract of *Cucumis melo Var. momordica*. showed remarkable anti-anxiety activity in the experimental subject.

Acknowledgement: The authors are grateful to the authorities of Shambhunath Institute of Pharmacy,Prayagraj for the facilities.

Conflict of interest: The authors declare no conflict of interest.

References:

1. Oliver Grundmann, Jun-Ichiro Nakajima , ShujiroSeo , VeronikaButterweck. Anti-anxiety effects of *Apocynum venetum* L. in the elevated plus maze test. Journal of Ethnopharmacology.2007; 110:406-411.
2. Yadav A V, Kawale LA, Nade V S. Effect of *Morus alba* L. (mulberry) leaves on anxiety in mice. Indian Journal of Pharmacology. 2008; (40): 32.
3. Masoumeh E, Mohammad K, Maryam FA. Coriandrum sativum: Evaluation of its anxiolytic effects on the elevated plus maze. Journal of Ethanopharmacology. 2005; 96:365-370.
4. Manokaran S, Jaswanth A, Sengottuvelu S et al. Hepatoprotective activity of *A. Lanata* Linn. against paracetamol induced hepatotoxicity in rats. Research J. Pharm. and Tech.2008; 1(4):398-400.
5. Muthukumaran P, Shanmuganathan P, Malathi C. Antioxidative and anti-microbial study of *A. lanata*. Asian Journal of Biochemical and Pharmaceutical research.2011; 1:2231-2560.
6. Ragavendran P, Arul Raj C, Sophia D et al. Elemental analysis of *A. lanata* (L.) by EDX method. International Research Journal of Pharmacy. 2012; 3(7):218-220.
7. Rajesh R, Chithra K, Parakh PM. *Aervalanata* (Linn) Juss. Ex Schult-An Overview, Indian journal of natural products and resources .2011; 2:5-9.
8. H. Gerhard Vogel (Ed.) Drug Discovery and Evaluation: Pharmacological assays. 2nd ed. Germany. Springer.2008; 622-626.
9. Rajesh Kumar Goel, Dilpreet Kaur, Priyanka Pahwa. Assessment of anxiolytic effect of nerolidol in mice. Indian J Pharmacol. 2016; 48(4): 450-452.
10. Jung YH, Ha RR, Kwon SH, Hong SI, Lee KH. Anxiolyticeffects of Julibroside solatedfromAlbizziajulibrissin in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* . 44, 184-192 (2013).
11. The Diagnostic and StatisticalManual of Mental Disorders (5th Edn) American Psychiatric Association, Washington, DC (2013).
12. Kunovac JL, Stahl SM. Future directions in anxiolyticpharmacotherapy. *Psychiatr. Clin. North. Am.* 18, 895-909 (1995).
13. Lader M, Morton S. Benzodiazepineproblems. *Br J. Addict.* 86, 823-828 (1991).
14. Doukkali Z, Bouidida H, Srifi A, et al. Anxiolytic plants in Morocco: Ethnobotanical and ethno-pharmacological study. *Phytothérapie* (2014).
15. Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM. Flora Europaea. Cambridge: Cambridge UniversityPress.Durand B. Le complexeMercurialisannua L. s.l. uneétudebiosystématique. *Annales. Des. Sciences. Naturelles. Botanique.* 12, 579-736 (1964).
16. Krahenbuhl M, Yuan YM, Kupfer P. Chromosome and breeding system evolution of the genusMercurialis (Euphorbiaceae): implications of ITS molecularphylogeny. *Plant Systematic and Evolution.* 234, 155-170 (2002).
17. Aquino R, Behar I, D'Agostino M, De Simone F, Schettino O. Phytochemical investigation on Mercurialisannua. *Biochem. System. Ecol.* 15, 667-669 (1987).
18. Novas ML, Wolfman C, JH, De Robertis E. Proconvulsantand 'anxiogenic' effects of n-butyl β carboline-3 carboxylate, an endogenousbenzodiazepinebindinginhibitorfrombrain. Pharmacol. Biochem. Behav. 30, 331-336 (1988).
19. OECD/OCDE. Guidelines for the testing of chemicals, reviseddraft guidelines 423; acute oral toxicity-acute toxic class method, OECD publishing (2002).
20. Sharma V, Gilhotra R, Dhingra D, Gilhotra N. Possible underlying influence of p38MAPK and NF-B in the diminishedantianxietyeffect of diazepam in stressedmice. *J. Pharmacol. Sci.* 116, 257-263 (2011).
21. Pellow S, Chopin P, File SE, Briley M. Validation of open closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J. Neurosci. Methods.* 14, 149-167 (1985).
22. Archer J. Tests for emotionality in rats and mice: a review. *Anim. Behav.* 21, 205-235 (1973).
23. .Siegel PS. A simple electronicdevice for the measurement of grossbodilyactivity of smallanimals. *Journal of Psychology.* 21, 227-236 (1946).
24. Gutiérrez-García AG, Contreras CM, Mendoza-López MR, Cruz-Sánchez S, García-Barradas O. A

single session of emotional stress producesanxiety in Wistar rats. *BehaviouralBrain Research*. 167, 30-35 (2006).

25. Lister RG. The use of plus-maze to measureanxiety in the mouse. Psychopharmacology. 92, 180-185 (1987).
26. .Belzung C, Griebel G. Measuring normal and pathologicalanxiety-likebehavior in mice: a review. *Behav. Brain. Res.* 125, 141-149 (2001).
27. Walf AA, Frye CA. (2007) The use of the elevated plus maze as an assay of anxiety-relatedbehavior in rodents. *Nature Protocols*. 2, 322-328.
28. Crawley J, Goodwin FK. Preliminary report of a simple animal behaviour for the anxiolyticeffects of benzodiazepines. *Pharmacol. Biochem. Behav.* 13, 167-170 (1980).
29. .Hall CS. Drive and emotionality: Factorsassociated with adjustment in the rat. *Journal of Comparative Psychology*. 17, 89-108 (1934)
30. Walsh RN, Cummins RA. The open-field test A critical review. Psychol. Bull. 83, 482-504 (1976).
31. Crawley JN. Exploratorybehaviourmodels of anxiety in mice. *Neurosci. Biobehav.* 9, 37-44 (1985).
32. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-likebehaviors: a review. *Eur. J. Pharmacol*. 463, 30-33 (2003).
33. Marder M, Viola H, Wasowski C, Wolfman C, Waterman PG. 6-Bromoflavone, a high affinity ligand for the central benzodiazepinereceptors is amember of a family of active flavonoids. *Biochemical and Biophysical Research Communications.* 223, 384-389 (1996).
34. Viola H, Wasowski C, Levi de Stein M, Wolfman C, Silveira R. A pigenin, a component of Matricariarecutitaflowers, is a central benzodiazepinereceptors–ligand with anxiolyticeffects. *Planta. Medica.* 61, 213-216 (1995).
35. Wolfman C, Viola H, Marder M, Wasowski C, Ardenghi P. Anxioselectiveproperties of 6,3'-dinitroflavone, a high affinitybenzodiazepinereceptor ligand. *Eur. J. Pharmacol.* 318, 23-30 (1996).

.