

Evaluation of Anxiolytic Activity and Chemical Characterization of Herbal Extract in Albino Rats

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ABSTRACT

The brain's ability to adapt, operate cognitively, and maintain health depends on neurogenesis and synaptic plasticity. A process known as neurogenesis involves the creation of new neurons. This process is most common in areas of the brain like the hippocampus that are involved in learning, memory, and emotional regulation. Neurogenesis is essential for preserving cognitive capacities and emotional stability into adulthood, contrary to previous beliefs that it only occurs during early childhood. Contrarily, synaptic plasticity allows brain connections to change in reaction to novel information and past experiences. Depression, anxiety, and neurodegenerative diseases like Alzheimer's have all been associated to disruptions in these systems. The promotion of brain function and resilience requires an understanding of the factors that impact neurogenesis and synaptic plasticity, such as aging, stress, exercise, and disease. How these pathways aid in mental health, emotional stability, and memory development is the subject of this research. It also delves into their possible use as treatment targets for issues related to neurological and mental health. Improving quality of life and long-term cognitive health may be possible as a result of novel treatments that improve brain function, assist in recovery from neurological impairment, and boost overall mental well-being. This could be made possible as our understanding of this field expands.

1. INTRODUCTION

The sophisticated network that enables the seamless regulation of internal environments and their relationship to the outside world is the CNS, that consist of brain and spinal cord and their connection with outside world. Neurodegenerative illnesses like Alzheimer's and Parkinson's as like as anxiety, depression, and epilepsy, may affect the central nervous system (Abed, 2023). We are actively seeking medicines that are effective, safe, and easily available since chronic disorders continue to have a large impact on global health and the economy. In this light, the integration of traditional knowledge with modern scientific research into herbal medicine's curative properties has become an exciting new area of study. Herbal medicines' pharmacological effects are due to the many bioactive components they contain, which include alkaloids, flavonoids, terpenoids, and saponins, all of which interact with different areas of the central nervous system. In line with the larger trend of preventative and customized treatment, there is a rising interest in herbal medicine, which is bolstered by the increasing tendency toward natural and integrative healthcare solutions (Balkrishna et al., 2024). Conducting behavioural tests, biochemical analysis, and electrophysiological research is all part of the investigation's rigorous experimental design. The anxiolytic, antidepressant, and locomotor effects of the extracts may be further understood by behavioural assays such the EPMT, forced swim test and open field test. Neuronal firing rates & synaptic activity are evaluated in electrophysiological investigations, while oxidative stress indicators, inflammatory mediators, and neurotransmitter levels are measured in biochemical analysis (MICHA, 2017).

2. MATERIAL AND METHODS

Plant material

Plant resources, such as *Delphinium denudatum* (*D. denudatum*) roots and *Amaranthus spinosus* (*A. spinosus*) leaves, were sourced from the botanical garden of IFTM University in Moradabad, Uttar Pradesh. Dr. Beena Kumari, a botanist from Hindu College in Moradabad, Uttar Pradesh, India, verified the authenticity of the *A. spinosus* leaves. Standard floras and herbarium data were used to ensure that the plant taxonomy was accurate (Siddique, 2025).

Preparation of plant extract

Preparing their extracts plant resources, such as *D. denudatum* roots and *A. spinosus* leaves, were sourced from the botanical garden of IFTM University in Moradabad, Uttar Pradesh. Dr. Beena Kumari, a botanist from Hindu College in Moradabad. After the medications were coarsely ground, they were extracted using a Soxhlet apparatus with petroleum ether at temperatures ranging from 60 to 80 degrees Celsius. This was followed by a hydro-ethanolic mixture consisting of 95% ethanol and 1:1 water. After removing the solvents by filtering and distillation, the extracts were dried using a rotatory vacuum evaporator (Maze, 2017).

Animals

Albino rats weighing 100-150g, subjected to a temperature humidity of 50-55%, and subjected to 12-hour light and dark cycles. Each polypropylene cage could only hold a maximum of three animals. During the course of the trial, the animals were provided with water and a normal meal. Under the consent of the institutional animal ethics committee of IFTM University, Moradabad. The study's animal experiments were carried out. Institutional review boards at IFTM University, Moradabad's College of Pharmacy, Pharmacy Department, and Institute for Animal Ethics all gave their stamp of approval to the research (Abid & Khan, 2017).

Drugs

Diazepam (standard drug) was purchased from Cipla Ahmedabad India and Tween 80 required for this study was purchased from CDH-Laboratory Reagent pvt. ltd. New Delhi, India.

Acute toxicity study

The five of which albino male, were given extracts of *D. denudatum* extract (DDE) and *A. spinosus* extract (ASE) at doses ranging from 5/50/300/2000 mg/kg, given orally using the fixed dose procedure 420. Following this, the animals were under continual observation for 1.5 hours at 4-hour intervals for any noticeable changes in behaviour up to 72 hours, and then for 14 days for any signs of death. The LD50 value was calculated at maximal medication of 2000 (mg/kg). Based on the LD50, a low dosage of 200 (mg/kg) and a high dose of 400 (mg/kg) were chosen, respectively, to conduct this investigation (Muñoz et al., 2021).

Anxiolytic activity

Experimental design

Group I: Normal control group be given 2% Tween 80 in distilled water (5 ml/kg, p.o.)

Group II: Anxiety control group be given 2% tween 80.

Group III: Be given DDE (200 mg/kg, p.o.)

Group IV: Be given DDE (400 mg/kg, p.o.)

Group V: Be given ASE (200mg/kg, p.o.)

Group VI: Be given ASE (400 mg/kg, p.o.)

Group VII: Be given combination (100 mg/kg of DDE + 100 mg/kg of ASE, p.o.) (C₁)

Group VIII: Be given combination (200 mg/kg of DDE + 200 mg/kg of ASE, p.o.) (C₂)

Group IX: Be given diazepam (2mg/mg i.p) (Li et al., 2007).

Methods

Elevated plus maze test

With 2 open arms measuring 5 × 10 cm and 2 closed arms measuring 5 × 10 × 4.5 cm, this EPM is arranged in a plus sign pattern. Forty centimetres was its height. The animal's head was cocked in the direction of the EPM's welcoming arms. Each five-minute interval, the duration of time consumed in the open area position was noted. When it entered the arena, the animal was required by regulation to have all four paws on its arm (Leo & Pamplona, 2014).

Dark and light test

Each animal was placed in its own designated spot in the middle of the device and tracked activity levels in both the light and dark chambers for five minutes each similar to EPMT, the animals were treated and grouped together. The duration of light area entries increases when anxiolytics are administered at dose, but this effect is not seen with non-anxiolytics (Ramos et al., 2008). It became gloomy due to a cover that separated a well-lit area from a section that occupied about one third of the cage (50 × 80 cm). A 20-centimeter-diameter circular aperture allows rats access to and from the compartment's lit and dark sections. Thirty minutes before to testing, the mice were given test extracts orally. To start the evaluation, the mouse was placed in the middle of the box's lit area. We calculated the percentage of time duration in the light chamber (Jiang et

al., 2023).

In vitro Antioxidant activity

DPPH Free radical test

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) test was carried out to find the free radicals scavenging limit of ethanolic concentrate of *A. spinosus* extract (ASE) and *D. denudatum* (Delphinium denudatum) extract (DDE). DPPH (200 µM) was prepared in a 95% methanol solution. Quantities of 25, 50, 75, 100, 125, and 150 µg/ml were extracted from the standard plant extract arrangement and placed in six separate test tubes (Hussen & Endalew, 2023). After 10 minutes of incubation with the test drug, 0.5 ml of the freshly constructed DPPH arrangement was measured for absorbance at 517 nm using a spectrophotometer. The reference used was standard ascorbic acid. The given formula was used to get the count % of DPPH free radicals (Baliyan et al., 2022).

$$\text{Statistical significance of scavenging (\%)} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The Mean±SEM is the computed result. The experimental groups were compared using Graph Pad Prism (Version 5.01), which included a one-way Analysis of Variance (ANOVA) followed by Dunnett's test. Statistical significance was determined when $p < 0.05$ (Rishikesh et al., 2017).

3. RESULTS

Determination of (%) yield

The yield percentages of 4.8% for DDE and 7.9% for ASE, when derived using hydroethanol as a solvent, are shown in table 4.1. The hydro alcoholic (Ethanol: distilled water) extracts that were produced had a semisolid consistency and a dark reddish brown colour.

Table 1 Percentage yield of DDE and ASE from 100 g of dried material

Extraction	Solvents Use	Materials	Extract Yield
DDE (Soxhlation)	Hydroethanol	Dried powdered root	4.8%
ASE (Soxhlation)	Hydroethanol	Dried powdered leaves	7.9%

Preliminary phytochemical study

Results showed that DDE included a variety of substances, including steroids, alkaloids, tannins, flavonoids, proteins, carbs, and sterols.

The ASE test medication included alkaloids, tannins, flavonoids, proteins, saponins, sterols, and steroids. A comprehensive analysis was conducted.

Estimation of total phenols

The results showed that 63.24 mg of GAE/g for DDE and 57.05 mg of GAE/g for ASE in terms of total phenolic content.

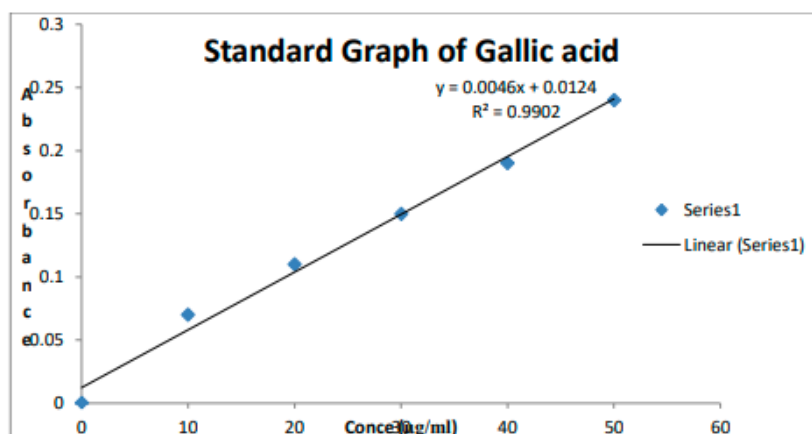


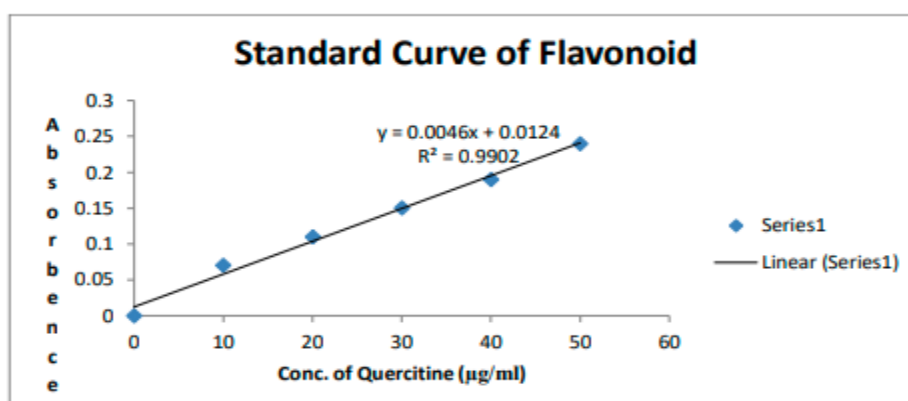
Figure 1 Standard graph of Gallic Acid

Table 2 Estimation of total phenols in DDE and ASE

Drug Extract	Total Phenols (mg of QE/g)
ASE	61.24±0.12
DDE	50.31±0.45

Estimation of total flavonoids

Total flavonoid concentrations of 31.23 mg of QE/g were detected in DDE, whereas 21.25 mg of QE/g were found in ASE, according to this assay (Pandey & Rajbhandari, 2015).

**Figure 1 Standard curve of Flavonoid****Table 1 Estimation of total flavonoids in DDE and ASE**

Drug Extract	Total Flavonoids (mg of QE/g)
ASE	30.27±0.02
DDE	20.25±0.25

Acute oral toxicity study

Study investigated the acute toxicity of DDE and ASE at doses reaching 2000 mg/kg.

The animal trials that included the extracts didn't show any signs of toxicity or mortality. Therefore, a maximum dose of 2000 mg/kg was used to establish the LD50 (Mbiri et al., 2023). This investigation used low dosages (200 mg/kg) and maximum dosages (400 mg/kg) based on fractions of LD50, respectively. Two doses of the two drugs were given in a 1:1 ratio, and a moderate dosage was chosen for the aim of the experimental studies (Chiranthan et al., 2022).

Anti-anxiety activity

In this experiment, rats were given oral doses of 200 and 400 mg/kg of DDE and ASE on the seventh day of treatment (Chiranthan et al., 2022). Table 4 shows that the anxiety control group was less affected by the first combination (C1 = 100 mg/kg of each medication) than by the second combination (C2 = 200 mg/kg of each drug), which had a very significant effect ($p < 0.001$).

Table 4: The effects of DDE, ASE, C1, C2 and diazepam in EPMT

S.N.	Groups	Dose (mg/kg), p.o.	Time spent in open arms (sec)
1	Control (vehicle)	5 ml/kg	27.50±4.40
2	Anxiety control AC	5 ml/kg	14.00±3.8

3	DDE	200mg/kg	22.00±3.4
4	DDE	400mg/kg	39.85±8.39
5	ASE	200mg/kg	32.00±7.45
6	ASE	400mg/kg	64.85±4.39
7	C ₁	100mg/kg	34.40±6.25
8	C ₂	200mg/kg	90.85±4.39
9	Diazepam	2mg/kg	87.00±2.82

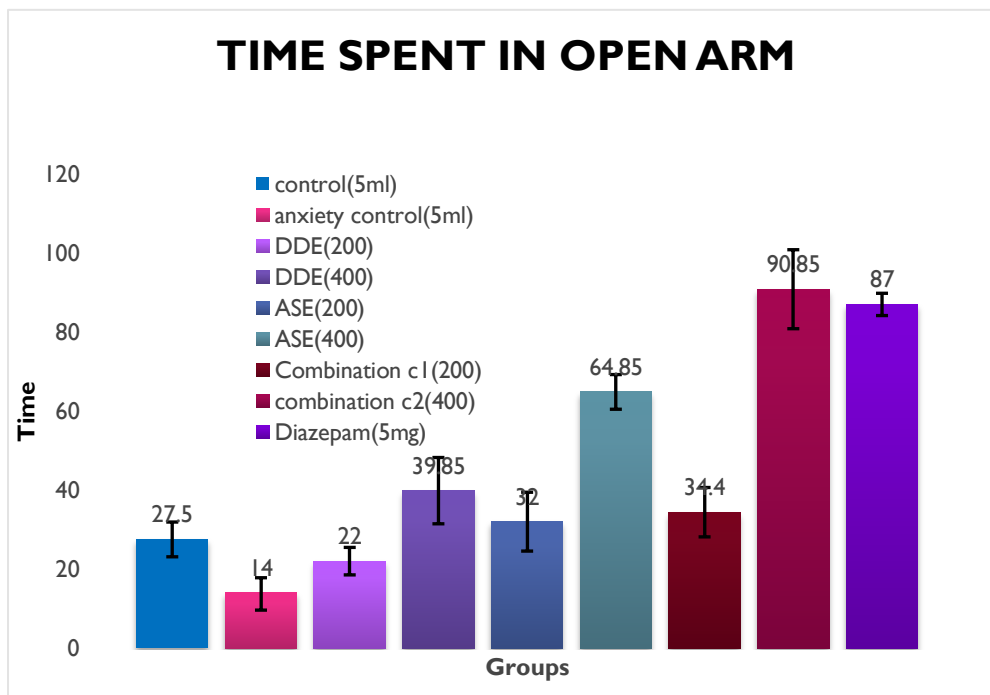


Figure 3: Effects of DDE, ASE, C₁, C₂ and diazepam in EPMT

Table 5 shows that the light and dark test did not reveal any remarkable effects in animals given 200 mg/kg of DDE, ASE, or combination C₁. However, there was a notable impact ($p < 0.05$) in animals given 400 mg/kg of DDE, and an even more noticeable reaction was seen in animals given 400 mg/kg of ASE. Last but not least, the anxiety control group spent much less time in the light chamber than rats given 2 mg/kg of diazepam or combination C₂ (Gopi et al., 2016).

Table 5: Effects of DDE, ASE, C₁, C₂ and diazepam

S.N.	Groups	Dose (mg/kg), p.o.	Time spent in open arms (sec)
1	Control (vehicle)	5 ml/kg	64.80±3.80
2	Anxiety control AC	5 ml/kg	34.80±9.40
3	DDE	200mg/kg	42.54±13.28
4	DDE	400mg/kg	59.85±8.39
5	ASE	200mg/kg	48.70±13.42

6	ASE	400mg/kg	74.45±4.09
7	C ₁	100mg/kg	39.40±16.05
8	C ₂	200mg/kg	113.55±11.09
9	Diazepam	2mg/kg	107.07±4.22

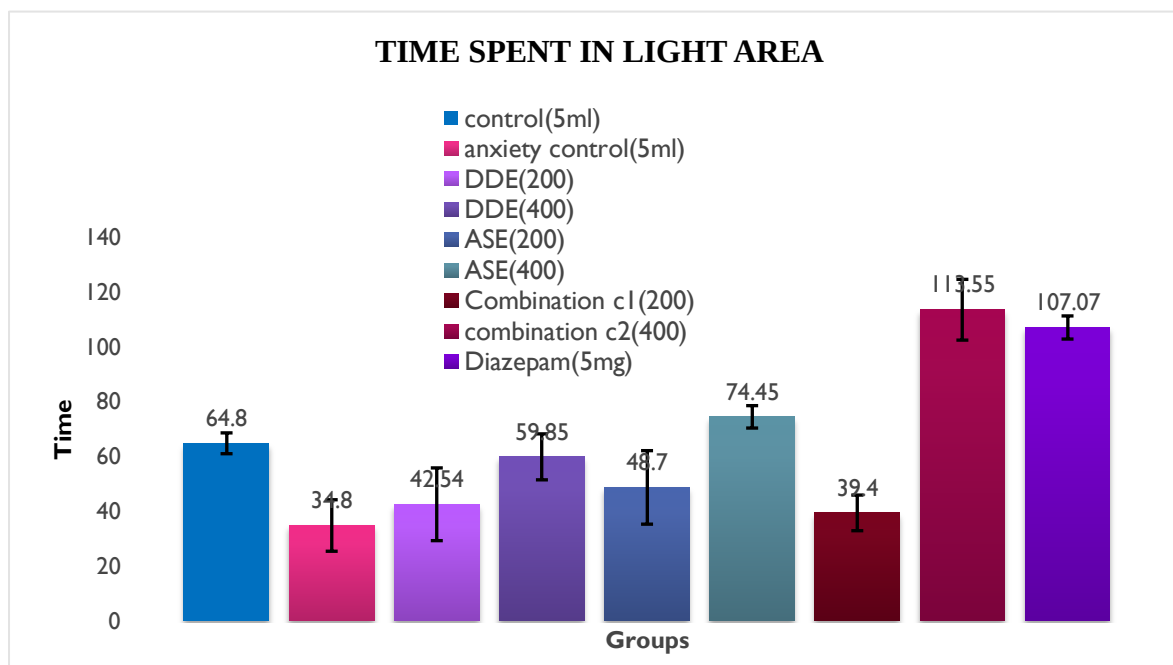


Figure: 5 Effects of DDE, ASE, C₁, C₂ and Diazepam in EPMT

4. DISCUSSION

Determination of percentage yield, preliminary phytochemical study, estimation of total phenols flavonoids, acute oral toxicity study selection of doses, anti-anxiety test by elevated + maze, light and dark model were all detected in the hydroalcoholic extract of the leaves of *Amaranthus spinosus*. An intriguing fact is that flavonoids, which include aglycone, glycosides, and methylated derivatives, make about half of these phenolic molecules (Abba et al., 2023). We find these phytochemicals in foods and natural remedies. In addition to their many other phenolic components, flavonoids have been found to be effective antioxidants, antibacterial, anti-inflammatory, immune system promoting, skin UV radiation protector, and potential pharmaceutical and medical uses (Herrera-Ruiz et al., 2008).

An acute toxicity test was performed on leaves extracts of *Amaranthus spinosus* and root extract of *Delphinium denudatum* at concentrations of up to 2000 mg/kg. The extracts did not show mortality or toxicity when tested on animals (Ambavade et al., 2006).

The majority of the time, animals in the EPM test choose to remain in the closed arms. This is an example of how animals' natural fear of open spaces manifests as an aversion to open arms. Animals treated with anxiolytic medications spend more time with their arms open, while those with anxiogenic properties spend more time with their arms folded (Ennaceur & Chazot, 2016).

5. CONCLUSION

Results show that an immediate dose of 400 mg/kg of an ethanolic extract of *Delphinium denudatum* root and *Amaranthus spinosus* leaves significantly reduces anxiety. The findings of this research highlight the importance of neurogenesis and synaptic plasticity in maintaining and improving brain function, especially in areas such as memory, learning, and emotional regulation.

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