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## Research Article

# Neuropharmacological Potential of a Northern Maidenhair Fern (*Adiantum pedatum*) L.

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## Abstract

**Background and Objective:** Perturbed neurological functions significantly affect the quality of life. Synthetic drugs may lead to improved neuronal function, however may seek to undesired effects. Hence, current study emphasized to determine the phytochemicals and antioxidant activity of *Adiantum pedatum* as well as to investigate the neuropharmacological potential of ethanolic extract of *Adiantum pedatum*. **Materials and Methods:** To determine neuropharmacological potential, four groups of 20 Swiss albino male mice were selected and labelled as control, standard (caffeine 10 mg/kg), low dose (200 mg/kg) and high dose (400 mg/kg) treatment groups. Neuropharmacological activities of plant extract were evaluated by performing in open field, rearing, cage crossed, head dip, traction and forced swimming test. One-way Analysis of Variance (ANOVA) was able to analyze the results of all used tests. *In vitro* anti-oxidant potential of *Adiantum pedatum* extract was investigated using DPPH assay, the phenolic contents were investigated. **Results:** The maximum phytochemicals were present in both chloroform and ethanol extracts. Results suggested that ethanolic extracts of *Adiantum pedatum* possess anxiolytic and CNS stimulant effect as an increased locomotor activity was observed during tests and also suggested the possible use of *Adiantum pedatum* in drug industry. **Conclusion:** Current study provides the neuroactive capacities of *A. pedatum* extract possibly through anti-oxidant means.

**Key words:** Phytochemicals, antioxidants, CNS stimulant, anxiolytic, drug, medicinal plant

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Brain disorders owing to compromised neuronal activity are considered as a serious public health issue. Today's stressful life events have been linked to the major psychiatric and depressive mental disorders such as anxiety, seizures, depression, Alzheimer disease, cerebrovascular impairment, parkinsonism and many others. The occurrence rate of neuropharmacological disorders is rising with each passing day<sup>1</sup>. Recently, World Health Organization (WHO) claims that people who either have or are at risk of mental disorders is now more than a billion. Depression and anxiety often present together and are the most serious progressive brain disorders. Depression is a serious and complex heterogeneous psychiatric disorder that can lead to long lasting disability all over the world<sup>2</sup>. Due to harmful effects of currently available anxiolytic drugs patients withdraw the treatment before full recovery<sup>3</sup>. One of the most widespread neurological diseases that affects people of all ages is epilepsy. In 2016, WHO presented a report according to which approximately 50 million people have epilepsy<sup>4</sup>. The most prevalent mental disorder today, Alzheimer's disease causes sleep and mood disturbances in addition to memory impairments, apathy, trouble remembering recent events and depression in its early stages<sup>5</sup>. The patients of these common neurological disorders have false beliefs and hallucinations like abnormal sensations<sup>6</sup>.

Oxidative stress is accompanied with more than 100 neurological conditions. Imbalance in reactive oxygen species (ROS) production besides cellular antioxidant capability causes oxidative stress<sup>7</sup>. To minimize oxidative stress natural antioxidants are required. Antioxidants are mainly isolated from plant origin and used for human neurobehavioral improvement<sup>8</sup>. Antidepressant drugs are used to treat brain related ailments which are easily available in the market but prolonged use of these drugs results in several adverse effects like drowsiness, mental impairment, sexual problem, urinary retention and cause different physiological, allergic and brain disorders<sup>9</sup>. Patients are losing hope as the danger of adverse reactions to antidepressant medications rises<sup>10</sup>. We can improve our mental health by consuming nutritional supplements which are rich in phytochemicals<sup>9</sup>. Currently available data suggest that several phytochemicals such as phenols or carotenes have valuable therapeutic properties and are found to be working against neurological disorders<sup>11</sup>. There is a critical need for natural alternative remedies that are more efficient and less hazardous. Plants are the primary source of these alternative

drugs<sup>10</sup>. The relationship between plants and human beings is as old as humankind itself<sup>12</sup>.

Plants are the natural resources of living gas on our planet. They provide oxygen gas that all living organisms breathe and utilize it in energy resource. The plant kingdom is regarded as a highly potential source of different drugs which are less expensive, easily available, safe, efficient and has less harmful effects<sup>13</sup>. It is a statistic that 25% of all prescriptions for medicines are based on compounds obtained from plants<sup>12</sup>. Different plants are used against CNS disorders as they possess a large number of new, active secondary metabolites and phytochemicals. These phytochemicals have antioxidant, antibacterial, analgesic, hypoglycemic, anti-inflammatory and radio protective activity and have been utilized in drug making since ages<sup>14</sup>. In plants, angiosperms are given more importance because they have more diverse adaptations, more biodiversity and are more widely dispersed while pteridophytes are over shadowed as they are less approachable to many research groups due to their limited distribution<sup>15</sup>.

Pteridophytes, one of the most ancient and lower vascular plant families, prefer wetness and shade and have a lengthy geological history<sup>16</sup>. Pteridophytes belong to one of the largest fern family Pteridaceae which is popular for its efficient pharmacological roles in the conventional medicines<sup>15</sup>. There are 250 distinct genera of ferns. Mankind has had more than 2000 years to become familiar with 12,000 species. They are an important component of flora next to angiosperms as they provide us with food, fiber, handicrafts, cosmetic formulations, building material, abrasives and ornamental decoration essentials. The ability of pteridophytes to withstand microbial ailments may be a factor in their evolutionary success<sup>17</sup>. The pteridophytes offer a great deal of therapeutic application in various disorders and are used in Homeopathic, Ayurvedic, Tribal and Unani medicines<sup>18</sup>. They exhibit antioxidant, anti-inflammatory, analgesic, antimutagenic, immunomodulatory and neuromodulatory activities<sup>15</sup>.

*Adiantum* is a commonly occurring genus of Adiantaceae family and is characterized by their delicate finely divided foliage. In Pakistan it is usually present in northern areas and Azad Jammu Kashmir<sup>19</sup>. *Adiantum* species produce bright yellow to orange colored spores. Its spores produce structures known as sporangia<sup>20</sup>. Several *Adiantum* species can be applied to treat the chest complaints, skin diseases, tumors of spleen, cough, increase in lactation, colds, fever, bronchial disorders and dandruff<sup>21</sup>. *Adiantum pedatum* is commonly known as "five fingered fern" and belongs to Pteridaceae

family. It can grow up to 2.5 feet tall. In spring fresh green fronds with attractive black shiny stem emerge from moist, fertile and humus rich soil to form a feathery clump<sup>22</sup>. It has fan shaped leaflets which bear yellowish orange spores. It is used by herbalists to cure kidney stone, nasal congestion, alopecia and urinary disorders. It is also used as central nervous system stimulant, demulcent, tonic and emmenagogue<sup>23</sup>.

The aim of this study was to evaluate the degree of neuropharmacological activity of (*Adiantum pedatum* L.) with response to caffeine concentration after application of some technical tests for male rats in the lab.

## MATERIALS AND METHODS

**Study area:** The healthy and fresh plants of *Adiantum pedatum* were collected from different areas of Khuratta, district Kotli, Azad Jammu Kashmir. The collection was done during the month of October and December, 2021. Plants were transported safely to the Government College University Lahore's Botany Laboratory where they were identified with voucher number (GC Herb Bot. 3086) (Fig. 1). The plant material was thoroughly washed under running water. Plants were allowed to dry in the shade for a week after washing. Because there was a danger that high temperatures would denaturize phytochemicals present in plants, this drying procedure was carried out away from the direct sunlight. The plants were processed through an electronic grinder to create a fine powder. This plant powder was kept at room temperature in airtight, dry plastic bag.

**Extract preparation of plant sample:** A 10% w/v solution of ethanol plant extract was prepared by adding 200 g of powder to 2000 mL of ethanol and shaking occasionally. The solution was placed in a glass beaker for 7 days at room temperature. In a large Petri plate, the filtrate was collected after 7 days of maceration using Whatman filter papers No. 1 and allowed to evaporate for 3-4 days at room temperature. After evaporation crude ethanolic extract was remained at the bottom of petri plate which weighed 1.74 g. Extract was stored in 50 mL falcon tube at 4°C until used for experiment.

**Fractionation of different solvents:** For fractionation in a separating funnel, ethanol extract was combined with various solvents like chloroform, n-hexane and water according to their polarity. The solvents used were chosen from nonpolar to polar in order of their polarity index.

**Phytochemical screening:** The presence of natural substances like proteins, alkaloids, glycosides, carbohydrates, saponins, flavonoids, phenols, tannins and amino acids were checked in plant extracts using following established techniques (Table 1).

**Evaluation of antioxidants:** Using the following biochemical procedures, the antioxidant activity was measured:

- DPPH radical scavenging activity
- Determination of total phenolic content

Table 1: Different chemical reactions used to determine the presence of phytoconstituents in different solvent extracts of *Adiantum pedatum* L.

Secondary metabolite	Name of test	Reactants	Expected result
Phenols	Ferric chloride test	1 mL of the filtrate+1 mL of 1% FeCl <sub>3</sub> <sup>24</sup>	Appearance of green or blue color
Flavonoids	Alkaline reagent test	5 mL of filtrate+equal volume of 20% NaOH <sup>25</sup>	Appearance of yellow color
Alkaloids	Mayer's test	2 mL of filtrate+2 mL of 1% HCl+kept in boiling water bath for 5 min+6-7 drops of Mayer's Wagner's reagent added to filtrate <sup>26</sup>	Appearance of creamish/red/orange/ brown precipitates
Cardiac glycosides	Keller-Kiliani test	2 mL of filtrate+1 mL of glacial acetic acid+3-4 drops of 5% FeCl <sub>3</sub> +1 mL of concentration H <sub>2</sub> SO <sub>4</sub> was added carefully to the solution <sup>25</sup>	Development of brown ring at the interface or the appearance of violet color
Carbohydrates	Molisch's test	2 mL of filtrate+2 mL of Molisch's reagent and shaken vigorously+2mL of concentration H <sub>2</sub> SO <sub>4</sub> was added carefully along the wall of the test tube <sup>25</sup>	Appearance of reddish ring at the junction of two liquids
Reducing sugars	Fehling's test	1 mL of filtrate+2 mL of Fehling's solution (A: 7% CuSO <sub>4</sub> in distilled water containing two drops of dil H <sub>2</sub> SO <sub>4</sub> ), B: 12% KOH and 35% sodium potassium tartrate in distilled water. Mixed A and B in equal amounts and boiled for 5 min <sup>27</sup>	Appearance of brick red precipitates
Tannins	Ferric chloride test	2 mL of filtrate+1 mL of 5% FeCl <sub>3</sub> <sup>28</sup>	Yellow brown precipitates
Saponins	Froth test	0.5 mL of the filtrate+0.5 mL of the distilled water and shaken vigorously for about 30 sec <sup>26</sup>	Formation of perseverance of froth
Proteins	Burette test	2 mL of the filtrate+1 mL of 40% NaOH+1-2 drops of CuSO <sub>4</sub> was added slowly to the solution <sup>28</sup>	Appearance of violet color showed the presence of peptide linkages in the solution
Amino acids	Ninhydrin test	5 mL extract+few drops of ninhydrin reagent <sup>28</sup>	Formation of purple color



Fig. 1: *Adiantum pedatum* L. herbarium sample

**Determination of total phenolic content:** According to a spectrophotometric assay previously reported, the total amount of soluble phenolics in each plant extract was measured<sup>29</sup>. As 9 mL of distilled water, 1 mL of the Folin-Ciocalteu reagent, 1 mL of the extract and 10 mL of Na<sub>2</sub>CO<sub>3</sub> were added to the mixture. By adding more distilled water, the total volume was then increased to 25 mL and well shaken. The mixture was given some time to cool to room temperature. A UV-visible spectrophotometer (UV-550; Jasco, Japan) was used to measure ABS (absorbance) at 724 nm after 40 min. Using a standard calibration curve created for various gallic acid concentrations, the total phenolics were quantified as micrograms of gallic acid equivalents (GAE) per gram of material.

**DPPH radical scavenging activity:** The activity of scavenging radicals was measured by the change in absorbance brought about by decreasing the DPPH radical. Based on the methodology given by Chu *et al.*<sup>30</sup>, this assay was conducted. In a nutshell, to prepare stock solution 24 mg of 1M DPPH was dissolved in 500 mL of methanol, wrapped in aluminium foil and allowed to sit at room temperature. By serially diluting standard or crude extract with methanol from a stock solution with a concentrations of 1 mg/mL, several concentrations (50 and 250 µL) were produced. As 1 mL of the crude plant extract or 1 mL of the standard, 3 mL of the DPPH solution and 1 mL of methanol made up the reaction mixture. Butylated Hydroxytoluene (BHT) was employed as the reference standard compound and 1 ml of methanol mixed with a 3 mL solution of DPPH served as the control. Methanol was used to create blank. The reaction mixture was then incubated for 30 min at room temperature in the dark. The absorbance value

was measured at 517 nm and the DPPH radical scavenging activity was calculated using the equation shown below:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where, A<sub>control</sub> is absorbance of DPPH and A<sub>sample</sub> is absorbance of sample.

**Study animal:** Adult male Swiss albino mice (25-30 g) were obtained and housed at animal housing facility of Department of Zoology, Government College University Lahore, Pakistan. They were housed to laboratory conditions for 1 week before experiments began. With 12 hrs light/dark cycles and standard temperatures and relative humidity, they were maintained in a climate controlled environment. Water and pellets were provided to the animals as part of their diet throughout the study duration. In order to conduct this investigation, 20 mice were split into 4 groups of 5 mice each. The following was the design and upkeep of the groups:

- Group 1:** Control group received 10 mL/kg water and a typical diet without any medication
- Group 2:** Caffeine (10 mg/kg dissolved in water, oral)
- Group 3:** 200 mg/kg dose of ethanolic *Adiantum pedatum* extract
- Group 4:** 400 mg/kg dose of ethanolic *Adiantum pedatum* extract

**Assessment of neuropharmacological activity:** Various assessments of mice's neuropharmacological activity,



Fig. 2: Oral administration of dose

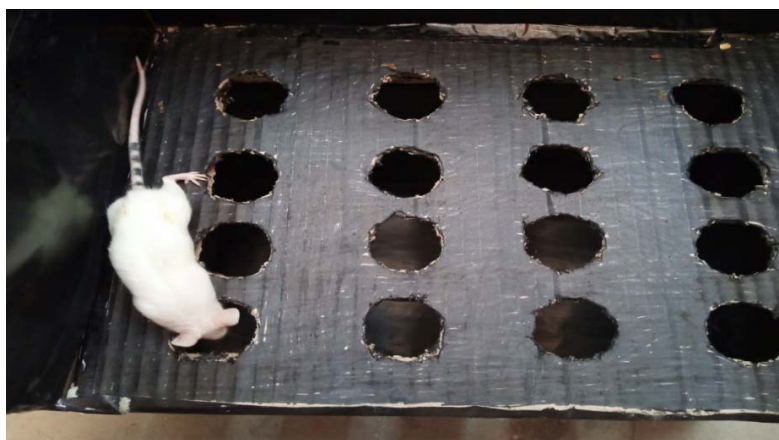


Fig. 3: Performance of head dip test

including different tests like open field, head dip, rearing, forced swimming, cage cross and traction which were made in a calm setting. These tests were performed to access the CNS stimulant effect of drug. For every test, each group was administered with respective drug dose through oral route and readings were taken right after 30 min of oral administration. The obtained results were statistically analyzed through One-way Analysis of Variance (ANOVA) test and Bonferroni *post hoc* test (Fig. 2).

**Head dip test:** Head dip test displays the mental state, learning ability and exploratory behavior of animals and usually used to evaluate anxiety and stress related disorders in animal models<sup>31</sup>. Head dip test was performed in an especially designed square shaped box (35×45×45 cm) which had a hole (2.5 cm in diameter) at each corner. This test is also called Shuttle Box<sup>32</sup>. All mice of four groups were placed each one in the center of this box for 10 mins and numbers of head dips or snout poking through these holes were calculated (Fig. 3).

**Rearing test:** In rearing test mice showed upward movement of their front limbs by keeping their body in erect position<sup>9</sup>. To carry out this test, a 1 L beaker was taken and covered its bottom with white paper. Inside this beaker, mice were placed and the number of times they moved upward while maintaining an upright posture was counted (Fig. 4).

**Cage cross test:** This examination measures locomotion and to screen sedative activity in mice<sup>33</sup>. It is carried out in a rectangular transparent plastic cage with sawdust covered floor which had a black colored partition in its center. This partition had a window which allowed the mice to cross the partition. Individually mice were placed in this cage and number of crossings through the partition was noted for 10 min Fig. 5(a, b).

**Traction test:** Mice were trained to move along a one-meter wooden pole so that the traction test could be performed.





Fig. 4: Performance of rearing test

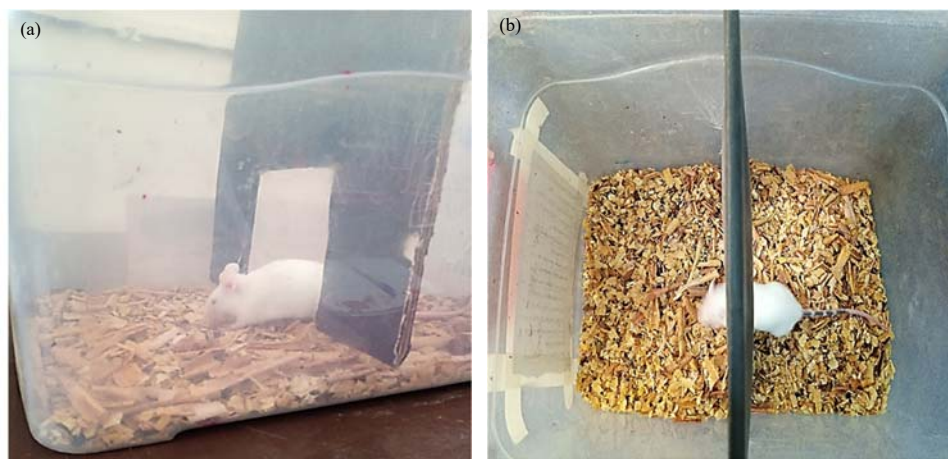


Fig. 5(a-b): Performance of cage cross test



Fig. 6: Performance of traction test

Using a stopwatch, the mice's movement from one end of the rod to the other was measured in seconds<sup>9</sup>. This test was used to determine whether a medicine

was stimulant or sedative, which could be determined by calculating an increase or decrease in this time (Fig.6).

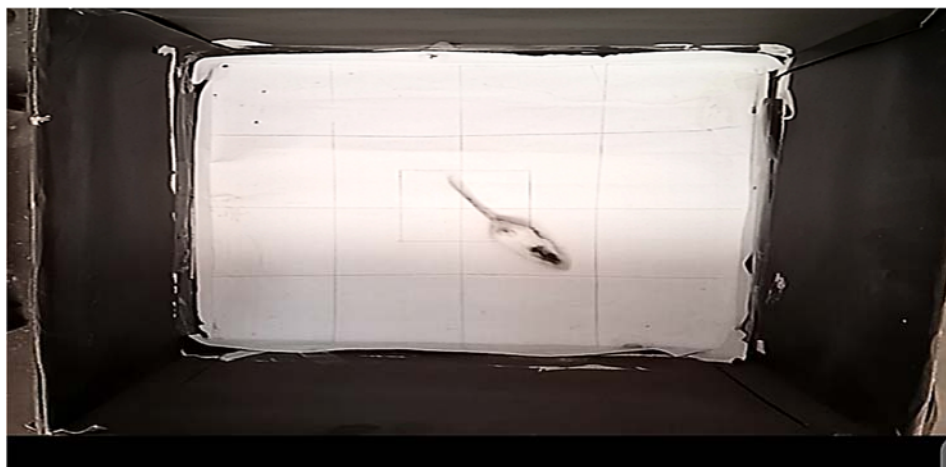


Fig. 7: Performance of open field test



Fig. 8: Performance of forced swimming test

**Open field test:** Open field test was fundamentally used to evaluate the locomotory activity of mice and performed in a specialized 50×50 cm box<sup>31</sup>. Inner lowered side of box was covered with white paper which was marked into 16 small squares with the help of lead pencil. Before the commencement of this test, a big black spot was made on the neck of each mouse with the help of permanent marker so that they could be easily detected in video. The camera was set on the camera stand in such manner that only open field setup completely visible in the camera. Following that, each mouse was removed from the cage and placed in the center of open field in a sequential order and a video of 5 min was recorded. Later on, these recorded videos were analyzed through ToxTrac software to calculate the distance covered by each mouse. Then manually the numbers of squares crossed by each mouse counted during 5 min (Fig. 7).

**Forced swimming test:** This experiment involved forcing mice to swim in a small, impenetrable space in order to assess the antidepressant activity of the mice test<sup>34</sup>. All mice were trained to swim before being put through the forced swimming test, which was conducted in a glass beaker with a 28 cm height and 16 cm diameter. At a temperature of roughly 25°C, the beaker was filled with water to the specified level. This test is called also Water-Maze<sup>35</sup>. In order to train them mice were placed one by one in the beaker for swimming for 6 mins. After training, mice were placed one by one in beaker and video of 6 min was recorded for each mouse. When mice moved quickly with their front and back paws, this was known as the “mobility time”, whereas when they moved very little except to stay floating, this was known as the “immobility phase” which indicated the state of despair. Then the mobility time is calculated manually by watching videos (Fig. 8).



**Ethical consideration:** This study was performed under ethical approval from Institute of Industrial Biotechnology, GC University Lahore, Pakistan with No. GCU-IIB-2230 after reporting that it followed. The US National Research Council's "Guide for the Care and Use of Laboratory Animals".

**Statistical analysis:** All the values were expressed as Mean $\pm$ SEM. The statistical significance of differences between the two means was assessed by One-way Analysis of Variance (ANOVA) was applied followed by a Bonferroni *post hoc* test. A difference at \*\* $p<0.01$  and \*\*\* $p<0.001$  were considered statistically significant and high significant, respectively<sup>36</sup>.

## RESULTS

### Antioxidant potential

**Total phenolic content:** Highest value was observed in aqueous fraction (52.72 $\pm$ 0.11) GAE followed by chloroform (37.24 $\pm$ 0.43), ethanol (20.66 $\pm$ 0.09) and n-hexane (22.61 $\pm$ 0.11) fraction, respectively. In comparison to a blank, the values for the total phenolic contents of the ethanol, n-hexane and chloroform fractions were deemed significant, whereas the value for the aqueous solution fraction was deemed non-significant. Gallic acid standard curve was calculated the results by determining the comparison and demonstrated the reference to GAE mg/mL (Table 2 and 3).

**DPPH free radical scavenging activity:** The BHT used as standard and DPPH potential obtained by different fractions was compared with it *Adiantum pedatum* L. n-hexane extract showed its greatest efficacy i.e. 68.54 $\pm$ 0.31 at a concentration

of 250  $\mu$ L. The range of 68.54 $\pm$ 0.31 to 20.75 $\pm$ 0.17 was found in different fractions of the extracts for the determination of DPPH potential. The concentration of the sample was found to be a factor in the (%) scavenging i.e. more the concentration of sample more the (%) inhibition (Fig. 9).

### Evaluation of neuropharmacological activity

**Cage cross test:** The cage cross test is used to investigate the neuroactive efficiency of drugs and bioactive compounds<sup>33</sup>. The mice in control group crossed the cage 27.6 $\pm$ 1.08 times. In caffeine treated group, mice traversed the cage 40.2 $\pm$ 2.13 times and was significantly ( $p<0.001$ ) higher comparing to control. Low dose of extract treatment (200 mg/kg) led to 37.2 $\pm$ 2.08 times frequency of cage cross and was significantly ( $p<0.01$ ) higher comparing to control. The mice in high dose extract treatment (400 mg/kg) group crossed the cage 50.8 $\pm$ 1.56 times which was significantly ( $p<0.001$ ) higher comparing to control. High dose extract showed maximum cage crossing activity (Fig. 10).

**Head dip test:** Since head dipping behavior is directly related to an individual's emotional state, the head dip test is used to investigate the anxiety-related behaviour of mice<sup>31</sup>. In control group the number of head dips was 20 $\pm$ 0.71. While the standard group showed the values 33 $\pm$ 1.56 for head dips which was significantly ( $p<0.001$ ) higher than control. Both low dose (200 mg/kg) and high dose (400 mg/kg) of extract treatment showed increased head dipping activity when compared with control. The values of head dips for low dose treated group was 29.4 $\pm$ 1.29 which was significantly ( $p<0.001$ ) higher comparing to control group of mice. The values for low dose treatment group were lower than

Table 2: Detection of phytochemical compounds in different plant extracts of *Adiantum pedatum* L.

Phytochemicals	Ethanol	n-hexane	Chloroform	Aqueous solution
Alkaloids	+	+	+	-
Carbohydrates	-	+	-	+
Cardiac glycosides	+	+	+	-
Flavonoids	+	+	-	-
Phenols	+	+	+	-
Proteins	+	-	+	+
Reducing sugars	-	+	+	-
Saponins	+	-	+	+
Tannins	+	-	+	-
Amino acids	-	-	-	-

Table 3: TPC evaluation of different solvent extracts of *Adiantum pedatum* L.

Solvent	GAE mg/mL $\pm$ SEM
Ethanol	20.66 $\pm$ 0.09
n-Hexane	22.61 $\pm$ 0.11
Chloroform	37.24 $\pm$ 0.43
Aqueous solution	52.72 $\pm$ 0.11

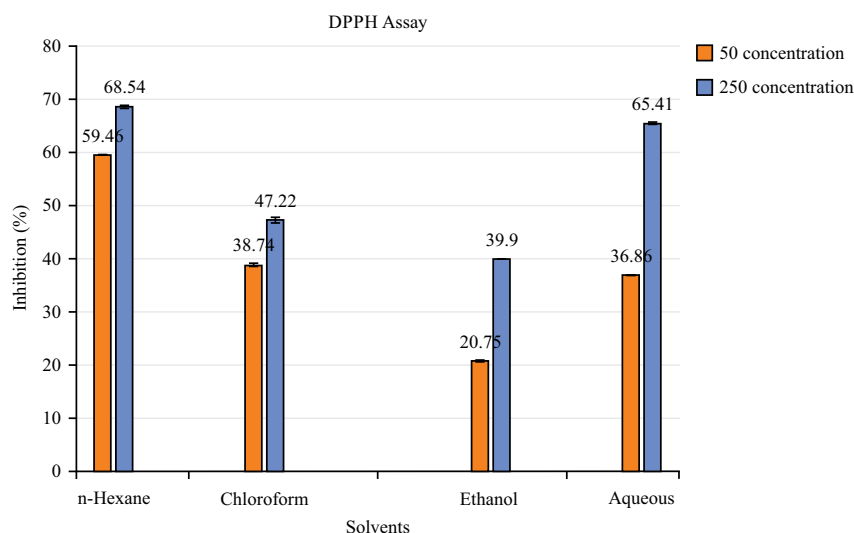


Fig. 9: DPPH free radical scavenging activity (%) of different solvent extracts of *Adiantum pedatum* L.

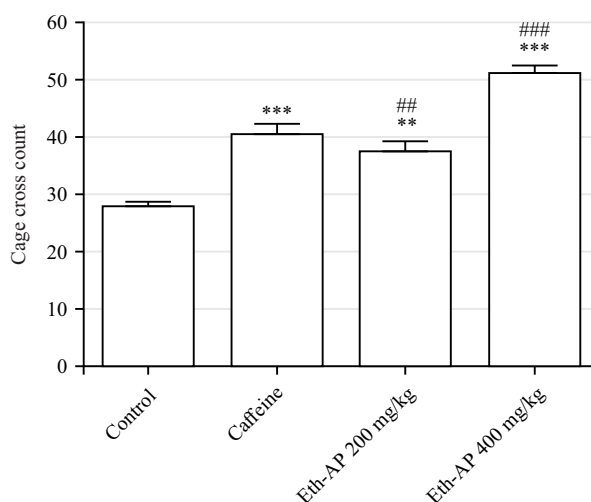


Fig. 10: Increased frequency of cage cross in extract treated mice

Mice in extract treated groups showed significant higher cage cross tendency comparing to control mice. Data represented as Mean ± SEM (n = 5 per group), \*\*p < 0.01, \*\*\*p < 0.001 vs Control, ##p < 0.01 and ###p < 0.001 vs Caffeine, One-way Analysis of Variance (ANOVA) was used then a Bonferroni post hoc test

the values of standard group. In high dose treated group mice showed  $39.6 \pm 1.50$  number of head dips which was significantly ( $p < 0.001$ ) higher than control (Fig. 11).

**Rearing test:** In rearing test mice showed upward movement of their front limbs by keeping their body in erect position<sup>9</sup>. During this test control group value was  $23.8 \pm 0.58$  while for caffeine treated standard group was  $34.2 \pm 1.53$ . The values of standard group were significantly ( $p < 0.001$ ) higher comparing to control group. Low dose treated group showed upward

movement for  $39.2 \pm 1.35$  times significantly ( $p < 0.001$ ) higher comparing to control. Similarly, the values for high dose treated group were  $47 \pm 0.84$  significantly ( $p < 0.001$ ) higher than control. These values showed increased mental activity of mice (Fig. 12).

**Traction test:** In traction test control group mice travelled one-meter wooden rod in  $9 \pm 0.32$  se. The mice of caffeine treated group travelled the rod in  $6.8 \pm 0.37$  sec significantly ( $p < 0.01$ ) lower than control group. The extract treated

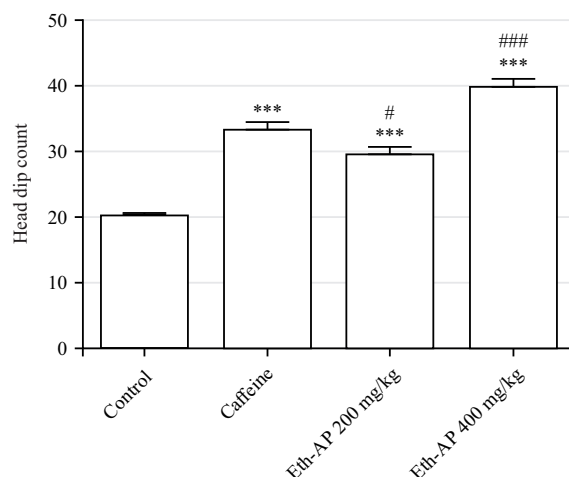


Fig. 11: Increased number of head dips in extract treated mice

Mice in extract treated groups showed significant higher number of head dips comparing to control mice, Data represented as Mean ± SEM (n = 5 per group), \*\*\*p<0.001 vs Control, \*p<0.05 and ###p<0.001 vs Caffeine, One-way Analysis of Variance (ANOVA) was used then a Bonferroni *post hoc* test

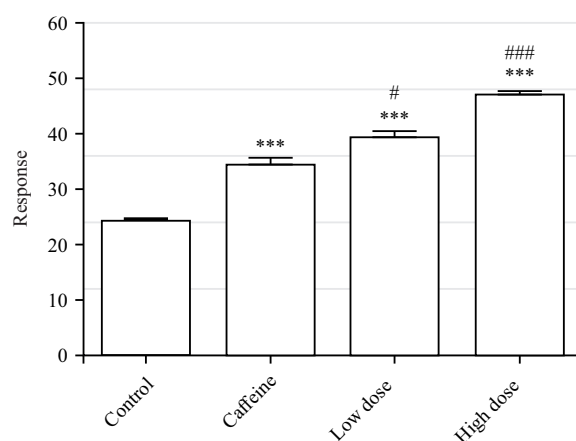


Fig. 12: Increased rearing activity of mice due to extract dose

Mice in extract treated groups showed increased upward movement of mice comparing to control mice, Data represented as Mean ± SEM (n = 5 per group), \*\*\*p<0.001 vs Control, \*p<0.05 and ###p<0.001 vs Caffeine, One-way Analysis of Variance (ANOVA) was used then a Bonferroni *post hoc* test

groups crossed the same rod in less time. Value of traction test for low dose extract treatment group was  $6 \pm 0.32$  significantly ( $p < 0.001$ ) lower comparing to control. The mice of high dose extract treated group consumed lowest time to cross rod and travelled the rod in  $5.2 \pm 0.37$  sec significantly ( $p < 0.001$ ) lower than control (Fig. 13).

**Forced swimming test:** Behavioral despair in mice is assessed using the forced swimming test<sup>1</sup>. In forced swimming test recorded mobility time for control group was  $216.2 \pm 4.48$  sec while in standard group duration of mobility time was recorded as  $257 \pm 3.44$  significantly ( $p < 0.001$ ) higher comparing to control. Low dose extract treated group showed mobility phase for  $262.6 \pm 2.53$  sec while high

dose extract treated group showed mobility phase for  $280.8 \pm 1.96$  sec. The values of both low and high dose extract treated groups were significantly ( $p < 0.001$ ) higher comparing to the value of control (Fig. 14).

### Open field test

**Total distance covered (m):** When animals are exposed to a new environment, it has been linked to emotionality in open field tests. A worried animal exhibits less usual activities, such as rearing and grooming and less ambulation<sup>31</sup>. The standard group (Fig. 15b) showed the mean  $11.09 \pm 0.54$  m distance covered in 5 min which significantly ( $p < 0.01$ ) higher as compared to control group (Fig. 15a) which showed  $7.69 \pm 0.45$  m distance covered in 5 min. Low dose extract

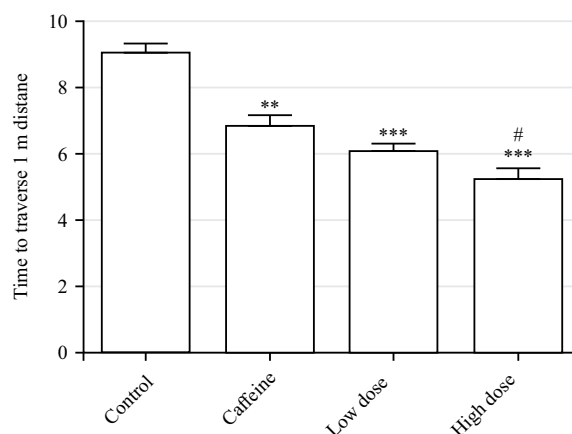


Fig. 13: Extract treated mice travelled the rod in minimum time

Compared to the mice in the control group, mice in the extract-treated groups took less time to cross the one-meter rod, Data represented as Mean  $\pm$  SEM (n = 5 per group), \*\*p<0.01, \*\*\*p<0.001 vs Control and #p<0.05 vs Caffeine, One-way Analysis of Variance (ANOVA) was used then a Bonferroni *post hoc* test

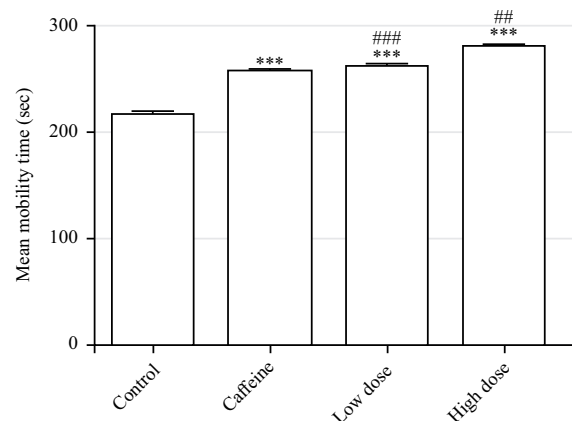


Fig. 14: Increased mobility time in extract treated group

Mice in extract treated groups remained in mobile phase for longer time as compared to control mice, Data represented as Mean  $\pm$  SEM (n = 5 per group), \*\*\*p<0.001 vs Control, #p<0.01 and ###p<0.001 vs Caffeine, One-way Analysis of Variance (ANOVA) followed by Bonferroni *post hoc* test

group (Fig. 15c) covered distance was  $13.03 \pm 0.54$  m which significantly (p<0.001) higher comparing to control. While high dose extract group (Fig. 15d) covered distance was  $17.43 \pm 0.55$  m which significantly (p<0.001) higher as compared to control. It can easily be seen that control group showed less locomotor activity while the standard and extract treated groups covered more distance in 5 min. Mice in extract treated groups showed increased upward movement of mice comparing to control mice. Data represented as Mean  $\pm$  SEM (n = 5 per group). \*\*\*p<0.001 vs Control, #p<0.05 and ###p<0.001 vs Caffeine. One-way ANOVA test followed by Bonferroni *post hoc* test (Fig. 15e).

**Total entries:** The control group showed the mean  $94.4 \pm 4.36$  number of entries while caffeine treated group showed

mean  $125.6 \pm 4.83$  number of entries which significantly (p<0.01) higher as compared to control group. In low dose and high dose extract treated group number of entries was  $136.4 \pm 3.27$ ,  $198.6 \pm 10.17$ , respectively which differed significantly (p<0.001) higher comparing to control group (Fig. 16).

## DISCUSSION

*Adiantum pedatum* L. is abundant in tropical and sub-tropical regions. Current study investigates its neuroactive potential on healthy adult mice. It agrees with the newest research that treated on stem bark of *Anopyxis klaineana* to examine the neuropharmacological effects<sup>37</sup> besides annual herbaceous cannabis plant<sup>38</sup>. The well-known neurostimulant

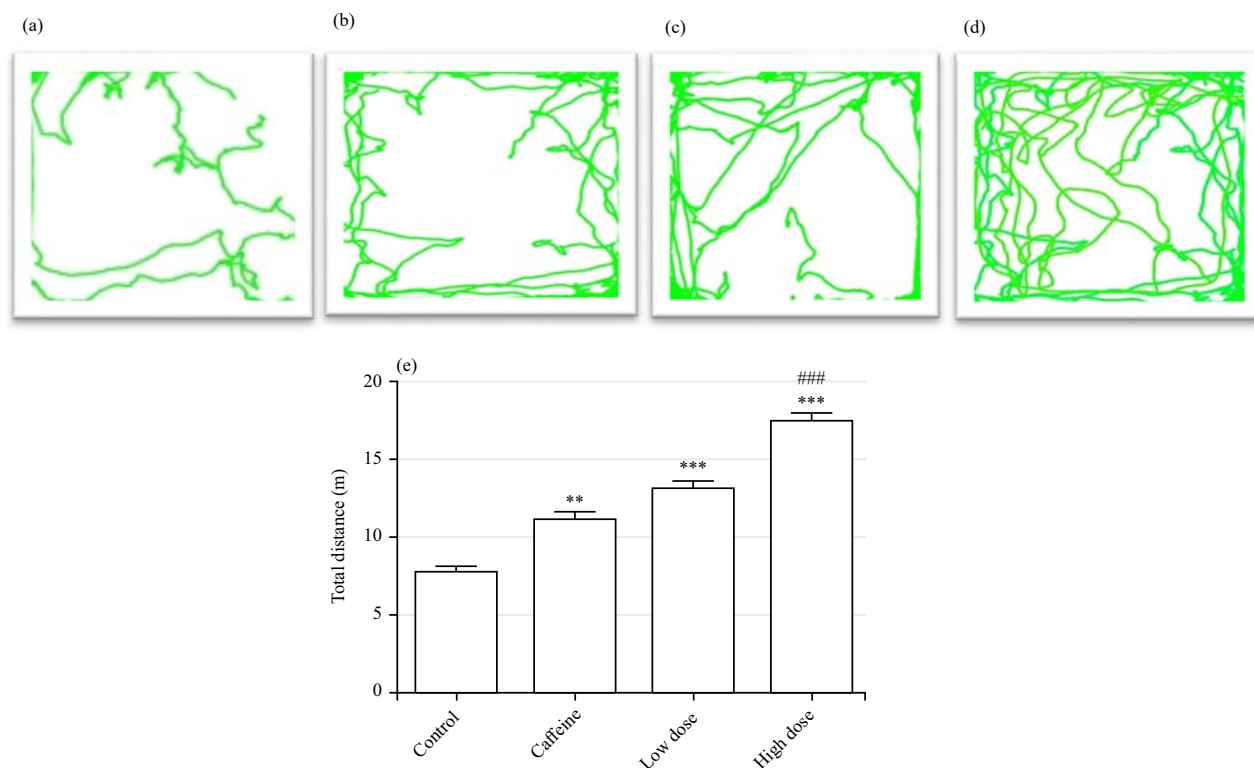


Fig. 15(a-e): In an open field test, extract treatment increased the locomotor activity of mice, Open field trajectories, (a) Control group, (b) Caffeine treated group, (c) Low dose extract treated group, (d) High dose extract treated group and (e) One way ANOVA test followed by Bonferroni *post hoc* test

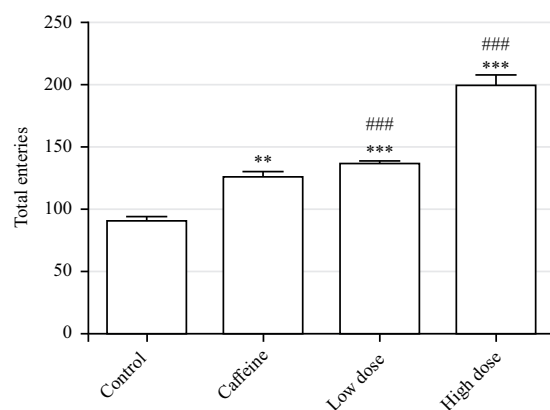


Fig. 16: Mice of extract treated group showed increased number of entries

Mice in extract treated groups showed increased number of entries in boxes as compared to control mice, Data represented as Mean  $\pm$  SEM (n = 5 per group), \*\*p<0.01 \*\*\*p<0.001 vs Control and ###p<0.001 vs Caffeine, One-way Analysis of Variance (ANOVA) followed by Bonferroni *post hoc* test

caffeine is used as standard drug to evaluate relative efficacy of *Adiantum pedatum* extract. Standard neurobehavioral assessment tests revealed a significant ( $p<0.05$ ) neurostimulatory potential of *A. pedatum* extracts with possible applications in brain-functioning impairments. Brain related ailments are a serious public health concerns in all across the world. Today's stressful life events have been linked

to the major psychiatric and depressive mental disorders including depression, stress, anxiety and many more<sup>4</sup>. Antidepressants can be used to treat these diseases, but patients are losing hope as a result of rising side effect risks linked with antidepressant medications and terminate the treatment before full recovery<sup>3</sup>. Therapeutics with neuroprotective and neuropharmacological activity that can



also enhance brain functions such as learning and memory are desperately needed<sup>39</sup>. There have been numerous reports of plants having effective against CNS diseases as they possess a surplus of novel and biologically active secondary metabolites and phytochemicals<sup>14</sup>. Due to their safe and productive outcomes, the focus of scientific study around the world has changed from synthetic medications to drugs based on botanical extracts. Usually angiosperms are used as source of herbal medicines and pteridophytes have been poorly studied by researchers for phytochemical analysis due to the habitat's sparse distribution and the local population's dwindling traditional unrecorded therapeutic knowledge<sup>15</sup>.

The goal of the present investigation was to determine the phytochemical components and antioxidant activity of *Adiantum pedatum* L. and whether mice could benefit neuropharmacologically from an ethanolic extract of *Adiantum pedatum* L. This investigation was evaluated by employing six behavioral tests i.e. open field, cage cross, traction, head dip, rearing and forced swimming test. These tests are classic and standard models for investigating the performance of central nervous system. The obtained results of these protocols were statistically analyzed through one-way ANOVA and Bonferroni *post hoc* test. A very limited research work has been done on this species regarding its phytochemical assay and medicinal value. Antioxidants are chemicals that can prevent or slow down oxidation by decreasing the amount of localized oxygen. From the last thirty years, natural antioxidants have drawn a lot of attention because synthetic antioxidants like BHT and BHA are now widely available on the market. The DPPH test is a popular technique for assessing the capacity of the natural antioxidants present in living cells to scavenge free radicals. At 517 nm, where DPPH exhibits its most intense absorbance, free radical scavenging is visible as a shift in the color from purple to yellow.

The phytochemical and antioxidant tests on *Adiantum pedatum* L. revealed remarkable results. According to the findings of the phytochemical analysis of all 4 extracts, phenols, proteins, saponins, flavonoids and carbohydrates were all present. Similar were reported by Živkovic *et al.*<sup>19</sup>. The action of DPPH was able to evaluate the potential radical scavenging activity at various concentrations. The *Adiantum pedatum* L. n-hexane extract showed its greatest efficacy at a concentration of 250  $\mu$ L. The range of  $68.54 \pm 0.31$  to  $20.75 \pm 0.17$  was found in different fractions of the plant extracts for the determination of DPPH potential. Highest TPC value was observed in aqueous fraction,

( $52.72 \pm 0.11$ ) GAE followed by chloroform ( $37.24 \pm 0.43$ ), ethanol ( $20.66 \pm 0.09$ ) and n-Hexane ( $22.61 \pm 0.11$ ) fraction respectively. For assessment of neuropharmacological potential different doses of extract were administered to mice orally.

Open field test was used to evaluate locomotor activity, in this study we found significant results in open field test as high dose extract treated group showed maximum locomotor activity as compared to control and caffeine treated group. In compliance with the current study findings Ruhul Amin *et al.*<sup>31</sup> and Kashkooe *et al.*<sup>34</sup> also reported similar results in *Vernonia anthelmintica* and *Dioscorea alata* respectively. In rearing test, the extract treated group showed significantly ( $p < 0.001$ ) higher values for struggling behavior or upward movement of mice as compared to control. These results are consistent with the reports of Kashkooe *et al.*<sup>34</sup> and Rubab *et al.*<sup>9</sup> in which there was a significant increase in struggling or rearing activity of mice were seen in mice treated with extracts of *Vernonia anthelmintica* and *Camellia sinensis* respectively. Traction test was used to determine the stimulant and muscle relaxant activity of mice. In this study it was observed that least time was taken by 400 mg/kg extract treated group as compared to control treated group which signified the stimulant activity of extract. Rubab *et al.*<sup>9</sup> and Ruhul Amin *et al.*<sup>31</sup> reported findings that were similar to those of the current investigation in *Camellia sinensis* and *Dioscorea alata*, respectively.

In forced swimming test mice showed mobile and immobile phase during 6 min. Mobility time in seconds was noted and considered for result analysis in forced swimming test which determine the stimulant effect of extract. When mice were treated with low and high doses of extract they showed significantly ( $p < 0.001$ ) higher duration of immobility comparing to control group. The result of this study is consistent with the reports of Sharma *et al.*<sup>40</sup> and Rubab *et al.*<sup>9</sup> in which there was a significant enhancement of motor activity in the mice treated with extracts of *Acorus calamus* and *Camellia sinensis*, respectively. The results of the animal's motor function and anxiolytic state were revealed by the head dip test. Increased number of head dips indicate the enhanced motor function and gives an indication of anxiolytic activity. When mice were treated with 200 and 400 mg/kg extract doses they showed increased head dipping behavior as compared to control. The results of present study are similar to the result reports of Ruhul Amin *et al.*<sup>31</sup> and Mahmudul Hasan *et al.*<sup>3</sup>, who worked for methanolic extract of *Dioscorea alata* and *Lygodium palmatum*, respectively and

observed increased head dipping activity of mice. Cage cross test is used to determine the exploratory and locomotory behavior of mice. Increase in number of cage cross counts indicate the mental activeness of mice. High dose group showed significantly ( $p < 0.001$ ) values for cage cross test as compared to control group which indicate the stimulant effect of plant extracts in mice. In compliance with the current study findings<sup>9,34</sup> also reported the same results in *Vernonia anthelmintica* and *Camellia sinensis*, respectively. Based on these findings, ethanolic extract of *Adiantum pedatum* may have anxiolytic properties and CNS stimulant effect as increased locomotor activity was observed during open field, cage cross, traction, head dip rearing and forced swimming tests. To determine the precise mode of action of the bioactive substances involved in neuropharmacological activity, comprehensively thorough phytochemical studies are strongly advised.

Ferns have been poorly studied by researchers for phytochemical analysis due to the habitat's sparse distribution and the local population's dwindling traditional unrecorded therapeutic knowledge. This research work will encourage the researchers to use phytoconstituents of *Adiantum pedatum* in medicines rather than synthetic chemicals. So, this study will be beneficial in manufacturing of natural herbal medicine for the treatment of brain linked impairments and malfunctions of such vital organs like reproductive organs.

### CONCLUSION

The phytochemical analysis confirmed the attendance of much more secondary metabolites like phenols, proteins, saponins, flavonoids and carbohydrates. Antioxidant tests on *Adiantum pedatum* also revealed remarkable outcomes. The results of this investigation indicate that the ethanolic extract of *Adiantum pedatum* may be neuropharmacologically active and safe for Swiss albino mice as model animals. It works as central nervous system stimulant and increase the locomotor activity of model animals. Further these findings suggested that *Adiantum pedatum* extracts might be used in the pharmaceutical business. Although the precise route of action for a plant's neuropharmacological activity has not yet been identified, it needs to be investigated.

### SIGNIFICANCE STATEMENT

By using natural products from plant resources to be alternative cures against disorder of nervous system as a general as well as anxiolytic and CNS stimulant additive manufacturing as a special besides improvement of living

behavior through measuring the reflexive attributes and reaction feedback. These plant resources should have stimulant ingredients which influence on living organisms without any side effects. Phyto-antioxidants besides phyto-phenols can enhance the impulses neuromuscular linkages and give positive results for neurology and behavior science. Pteridophytes are among natural resources serve in solving the neurobehavioral problems like (*Adiantum pedatum* L.). It can be representative as a good source of natural drugs. Emphasis of making use of all natural resources from higher and lower organisms and leaving all artificial and semi-artificial materials to decline the degree of pollution that our planet immersed in it.

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