Integrated phytochemical, computational, in vitro, and in vivo evaluation of the anxiolytic potential of *hamelia patens* jacq. Methanolic leaf extract

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Abstract
Anxiety disorders are among the most prevalent psychiatric conditions worldwide and represent a major public health concern due to their increasing incidence and associated socioeconomic burden. Although conventional anxiolytic drugs such as benzodiazepines are effective, their long-term use is often limited by adverse effects, tolerance, and dependence, highlighting the need for safer therapeutic alternatives. The present study aimed to evaluate the anxiolytic potential of the methanolic leaf extract of *Hamelia patens* Jacq. using an integrated in silico, in vitro, and in vivo approach. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, glycosides, tannins, saponins, triterpenoids, and carbohydrates. Molecular docking studies against the GABA_A receptor (PDB ID: 6D6T) demonstrated favourable binding interactions of the identified phytoconstituents, with apigenin exhibiting the highest binding affinity among the tested compounds. The extract also exhibited concentration-dependent GABA-transaminase inhibitory activity (IC_{50} = 112.6 ± 3.4 µg/mL) and significant antioxidant activity in the DPPH free radical scavenging assay (IC_{50} = 94.8 ± 2.8 µg/mL), suggesting its potential to enhance GABAergic neurotransmission and reduce oxidative stress. The anxiolytic activity was further evaluated in caffeine-induced Swiss albino mice using validated behavioural models, including the Elevated Plus Maze, Open Field Test, Y-Maze, and T-Maze. Oral administration of the methanolic extract at doses of 100, 200, and 400 mg/kg produced significant ($p < 0.05$–0.001) dose-dependent improvements in anxiety-related behavioural parameters compared with the negative control group. The 400 mg/kg dose exhibited anxiolytic efficacy comparable to diazepam, as evidenced by increased open-arm exploration, enhanced locomotor and exploratory behaviour, improved spontaneous alternation, and increased time spent in the goal arm. Collectively, these findings demonstrate that the methanolic leaf extract of *Hamelia patens* possesses significant anxiolytic activity, which may be mediated through modulation of the GABAergic system, inhibition of GABA-transaminase, and antioxidant mechanisms. The study provides scientific evidence supporting the therapeutic potential of *Hamelia patens* as a promising natural source for the development of novel anxiolytic agents. However, further studies on bioactive compound isolation, pharmacokinetics, chronic toxicity, and clinical evaluation are warranted to establish its therapeutic applicability.

Keywords: *Hamelia patens* Jacq.; anxiety; anxiolytic activity; GABA_A receptor; GABA-transaminase; molecular docking; DPPH assay; phytochemicals; medicinal plants.

1. Introduction

1.1 Anxiety Disorders

Anxiety disorders constitute one of the most common categories of psychiatric illnesses and are recognized as a major contributor to the global burden of mental health disorders. These conditions are characterized by persistent and excessive feelings of fear, apprehension, and worry that exceed normal responses to stressful situations. Individuals with anxiety disorders often experience a combination of psychological, behavioural, and physical symptoms, including nervousness, muscle tension, disturbed sleep, impaired concentration, palpitations, and excessive sweating. While anxiety serves as a normal protective mechanism against potential threats, prolonged or uncontrolled anxiety can significantly impair social interactions, occupational performance, and overall quality of life.

Image no.1: Types of Anxiety

The prevalence of anxiety disorders has increased considerably in recent decades, affecting individuals of all age groups irrespective of socioeconomic status. Epidemiological evidence indicates that women are more frequently affected than men, and many cases begin during adolescence or early adulthood. Despite advances in psychiatric care, a considerable number of patients remain inadequately treated because of delayed diagnosis, limited healthcare access, treatment costs, poor adherence, and the social stigma associated with mental illness. Consequently, anxiety disorders continue to represent an important challenge for clinicians and researchers seeking more effective and safer therapeutic interventions.

The development of anxiety disorders is associated with complex interactions among genetic, environmental, and neurochemical factors. Disturbances in neurotransmitter systems, particularly the γ-aminobutyric acid (GABA), serotonin, dopamine, and norepinephrine pathways, play a critical role in disease pathogenesis. Among these systems, GABA is the principal inhibitory neurotransmitter in the central nervous system and is essential for regulating neuronal excitability. Reduced GABAergic activity has been strongly associated with increased anxiety, making the GABAergic pathway an important target for anxiolytic drug development.[1-4]

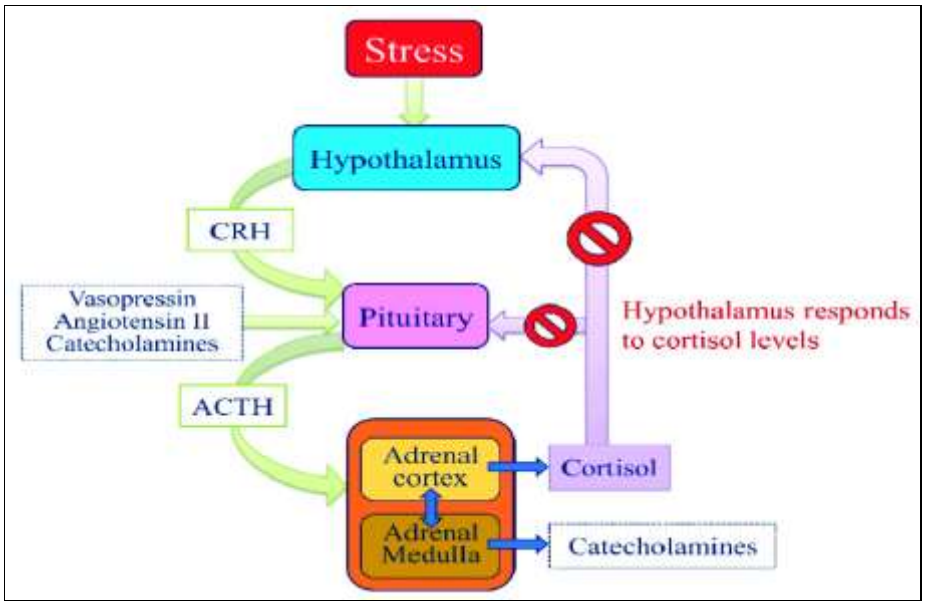


Image no.2: Hypothalamic–Pituitary–Adrenal (HPA) Axis Regulating the Stress Response

1.3 Herbal Medicine as a Source of Novel Anxiolytic Agents

Medicinal plants have served as valuable therapeutic resources for centuries and continue to play an important role in healthcare worldwide. Herbal medicines contain a diverse range of bioactive phytochemicals that can influence multiple biological pathways simultaneously, making them attractive candidates for the management of complex disorders such as anxiety. Compared with many synthetic drugs, plant-derived preparations are often considered to have a lower incidence of adverse effects and better patient acceptance, although their efficacy and safety require scientific validation. [5–8].

Phytochemicals including flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, and glycosides have demonstrated promising neuroprotective and anxiolytic properties in experimental studies. These compounds may exert beneficial effects by modulating GABAergic and serotonergic neurotransmission, reducing oxidative stress, suppressing neuroinflammation, and protecting neurons from cellular damage. As interest in complementary and alternative medicine continues to expand, medicinal plants are increasingly being investigated as potential sources of safer anxiolytic agents. [7–10].

1.4 *Hamelia patens*

Hamelia patens Jacq., a member of the Rubiaceae family, is an evergreen medicinal shrub commonly known as Firebush. The species is widely distributed throughout tropical and subtropical regions and has long been utilized in traditional medicine for the treatment of wounds, inflammatory conditions, microbial infections, fever, pain, and various skin disorders. [11–13]. Recent phytochemical investigations have revealed that the plant contains numerous secondary metabolites, including flavonoids, phenolic acids, alkaloids, tannins, saponins, terpenoids, and glycosides, many of which possess significant pharmacological activities. [11–13]

Experimental studies have demonstrated antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, analgesic, wound-healing, and antidiabetic properties of *Hamelia patens*. In particular, the presence of flavonoids and phenolic constituents suggests that the plant may also exhibit neuroprotective and anxiolytic effects. These bioactive compounds are known to interact with neurotransmitter systems associated with emotional regulation while simultaneously reducing oxidative stress, a process increasingly recognized as an important contributor to anxiety-related disorders.[11,12]

1.5 Research Gap

Although *Hamelia patens* has been extensively investigated for several pharmacological activities, relatively little scientific information is available regarding its potential role in the management of anxiety disorders. Most published studies have focused primarily on phytochemical characterization and biological activities such as antioxidant, antimicrobial, anti-inflammatory, and wound-healing effects, whereas systematic investigations of its anxiolytic properties remain limited. [11–15].

Furthermore, only a few studies have explored the interaction of the plant's phytoconstituents with molecular targets involved in anxiety, including the GABAergic system and GABA-transaminase enzyme. Comprehensive investigations integrating phytochemical analysis, computational molecular docking, biochemical assays, antioxidant evaluation, and validated behavioural models are therefore required to establish the anxiolytic potential of *Hamelia patens* and to clarify its possible mechanisms of action. [9,10,12].

2. Materials and Methods

2.1 Plant Material

The present investigation was carried out using the leaves of *Hamelia patens* Jacq. (Family: Rubiaceae), a medicinal plant traditionally used for the treatment of various ailments. The leaves were selected because of their reported phytochemical richness and potential neuropharmacological activity. The methanolic leaf extract served as the test material for all in vitro, in silico, and in vivo investigations. [16,17]

2.2 Preparation of Plant Extract

Fresh leaves of *Hamelia patens* were washed thoroughly to remove adhering dust and other contaminants, followed by shade drying at room temperature for approximately two weeks. The dried leaves were pulverized into a coarse powder using a mechanical grinder. About 500 g of powdered material was subjected to extraction by maceration using methanol (analytical grade) with occasional stirring for 72 h at room temperature. The extract was filtered through Whatman No. 1 filter paper, and the marc was re-extracted twice under identical conditions to ensure complete extraction of bioactive constituents. The combined filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at temperatures below 45°C to remove the solvent. The resulting semisolid extract was further dried in a vacuum desiccator, weighed to determine percentage yield, transferred to airtight amber-coloured containers, and stored.[18]

Image No.3: extraction process of hamelia patens

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the methanolic leaf extract was performed using established chemical methods to identify the presence of major classes of secondary metabolites. The extract was screened for alkaloids, flavonoids, phenolic compounds, tannins, saponins, carbohydrates, proteins, glycosides, and sterols based on characteristic colour reactions or precipitate formation. The results provided preliminary information regarding the phytochemical composition of the extract. [18,19]

2. 4Molecular Docking Study

An in silico molecular docking investigation was carried out to predict the interaction between selected phytoconstituents of *Hamelia patens* and the GABA $_A$ receptor. The three-dimensional crystal structure of the receptor (PDB ID: 6D6T) was obtained from the Protein Data Bank and prepared by removing water molecules and co-crystallized ligands. Ligand structures were generated and optimized using the MMFF force field prior to docking. Docking simulations were performed using V-Life Molecular Design Suite (Version 4.4–4.6). Binding affinity, docking score, hydrogen bonding, and hydrophobic interactions between ligands and receptor were analysed and compared with those of the standard anxiolytic drug. [20,21]

2.5 GABA-Transaminase (GABA-T) Inhibition Assay

The inhibitory activity of the methanolic extract against GABA-transaminase was evaluated using a spectrophotometric method. Different concentrations of the extract (25–400 µg/mL) were incubated with GABA substrate, α-ketoglutarate, phosphate buffer (pH 8.6), and GABA-transaminase enzyme under controlled conditions. Following incubation, $NADP^+$ was added to initiate the enzyme reaction, and absorbance was measured at 340 nm using a UV–Visible spectrophotometer. Vigabatrin served as the reference inhibitor. The percentage inhibition of enzyme activity and IC_{50} values were calculated to determine the inhibitory potential of the extract. [22,23]

2.6 DPPH Free Radical Scavenging Assay

The antioxidant activity of the methanolic extract was determined using the DPPH radical scavenging assay. Various concentrations of the extract (25–400 µg/mL) were mixed with freshly prepared DPPH solution and incubated in the dark at room temperature for 30 min. The decrease in absorbance was recorded at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference antioxidant. Radical scavenging activity was expressed as percentage inhibition and IC_{50} values. [24]

2.7 Experimental Animals

Healthy adult Swiss albino mice of either sex weighing 20–30 g were used for the behavioural studies. The animals were maintained under controlled laboratory conditions at a temperature of 27 ± 2°C with a 12 h light/12 h dark photoperiod and relative humidity of 55–65%. Standard laboratory pellet feed and purified drinking water were provided ad libitum. Before commencement of the study, the animals were acclimatized to the laboratory environment for one week. All experimental procedures were approved by the Institutional Animal Ethics Committee and were conducted in accordance with CPCSEA guidelines for laboratory care ,use.[25]

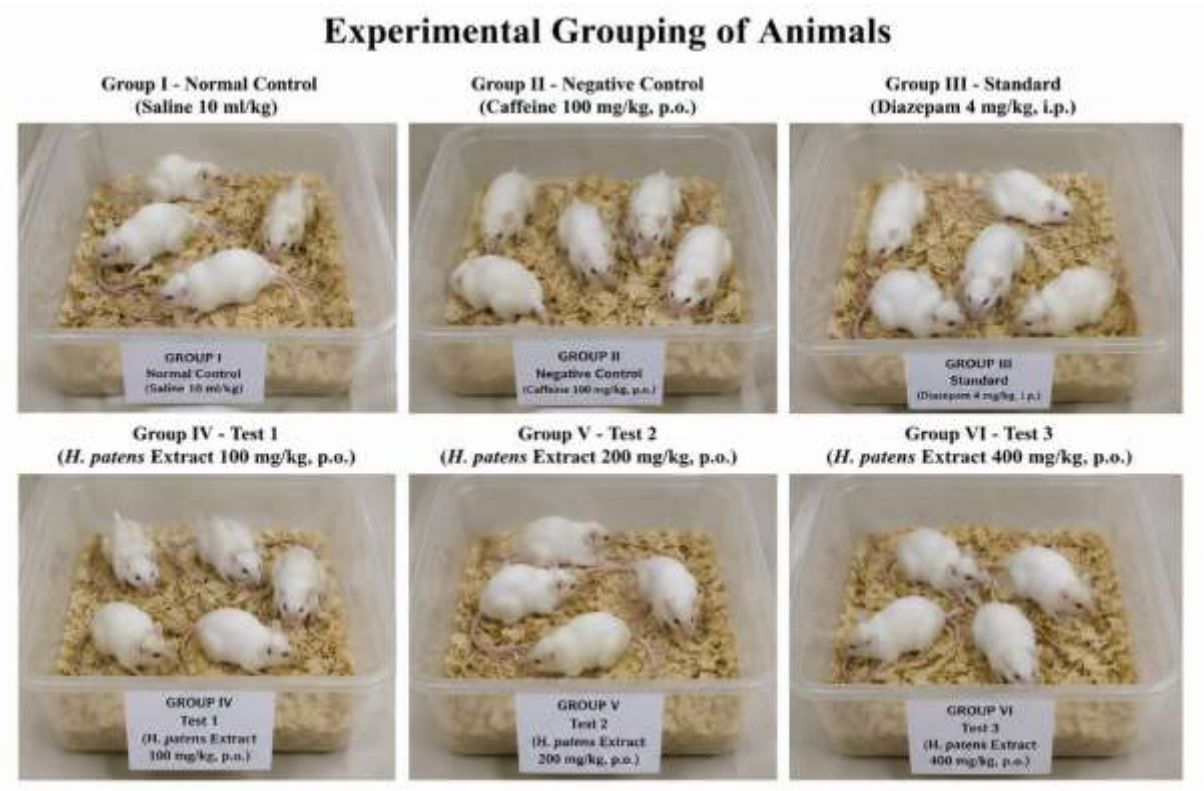


Image No. 4: experimental grouping of animals

2.8 Experimental Design

The animals were randomly allocated into six experimental groups consisting of six animals in each group. Anxiety-like behaviour was induced by oral administration of caffeine (100 mg/kg) once daily for seven consecutive days in all groups except the normal control. Following anxiety induction, treatment was initiated on the eighth day. The standard group received diazepam (4 mg/kg), while the treatment groups received methanolic extract of *Hamelia patens* at doses of 100, 200, and 400 mg/kg, respectively. Behavioural evaluations were performed 30 min after administration of the standard drug or plant extract using validated animal models of anxiety. [26,27]

2.9 Elevated Plus Maze Test

The Elevated Plus Maze was employed to evaluate anxiety-related behaviour in mice. The apparatus consisted of two open arms and two enclosed arms connected by a central platform. Thirty minutes after treatment, each animal was placed individually at the centre of the maze facing a closed arm and allowed to explore freely for 5 min. The number of entries into the open and closed arms together with the time spent in each arm were recorded. Enhanced exploration of the open arms was interpreted as evidence of anxiolytic activity. [28]

2.10 Open Field Test

Locomotor and exploratory behaviour were assessed using the Open Field Test. Each animal was placed at the centre of the arena and observed for 5 min. The number of squares crossed, rearing frequency, grooming behaviour, and duration of time spent in the central area were recorded. Increased locomotor activity and greater exploration of the central zone were considered indicative of reduced anxiety. [29]

2.11 Y-Maze Test

Spatial working memory and exploratory behaviour were evaluated using the Y-maze apparatus. Following treatment, each mouse was allowed to move freely within the maze for 5 min. The sequence of arm entries was recorded to calculate spontaneous alternation behaviour. Total arm entries, spontaneous alternation percentage, and time spent in the arms were analysed as behavioural parameters. [30]

2.12 T-Maze Test

The T-maze was used to evaluate exploratory behaviour and cognitive performance. Each mouse was introduced into the start arm and permitted to explore the maze for 5 min. The total number of arm entries, spontaneous alternation behaviour, correct arm choices, and time spent in the goal arm were recorded. Improved alternation behaviour and increased exploration were considered indicative of anxiolytic activity. [31]

3.Results

3.1Phytochemical Analysis:

Table no.1: Phytochemical analysis of methanolic Extract of *hamelia patens*

Sr. No.	Phytochemical Test	Expected Observation	Result
1	Alkaloids (Dragendorff's test)	Orange-red or reddish-brown precipitate	+
2	Glycosides (Keller-Killiani test)	Brown ring at interface with bluish-green upper layer	+
3	Flavonoids (Lead acetate test)	Yellow precipitate	+
4	Phenols (Ferric chloride test)	Blue-green, violet, or dark coloration	+
5	Triterpenoids (Vanillin–Sulfuric acid test)	Red, pink, or purple coloration	+
6	Tannins (Ferric chloride test)	Blue-black or greenish-black coloration	+
7	Saponins (Foam test)	Persistent froth/foam formation	+
8	Phytosterols (Salkowski test)	Reddish-brown ring/chloroform layer coloration	–
9	Carbohydrates (Molisch test)	Violet or purple ring at interface	+

Note:

(+) Presence of phytochemical constituents

(-) Absence of phytochemical constituents

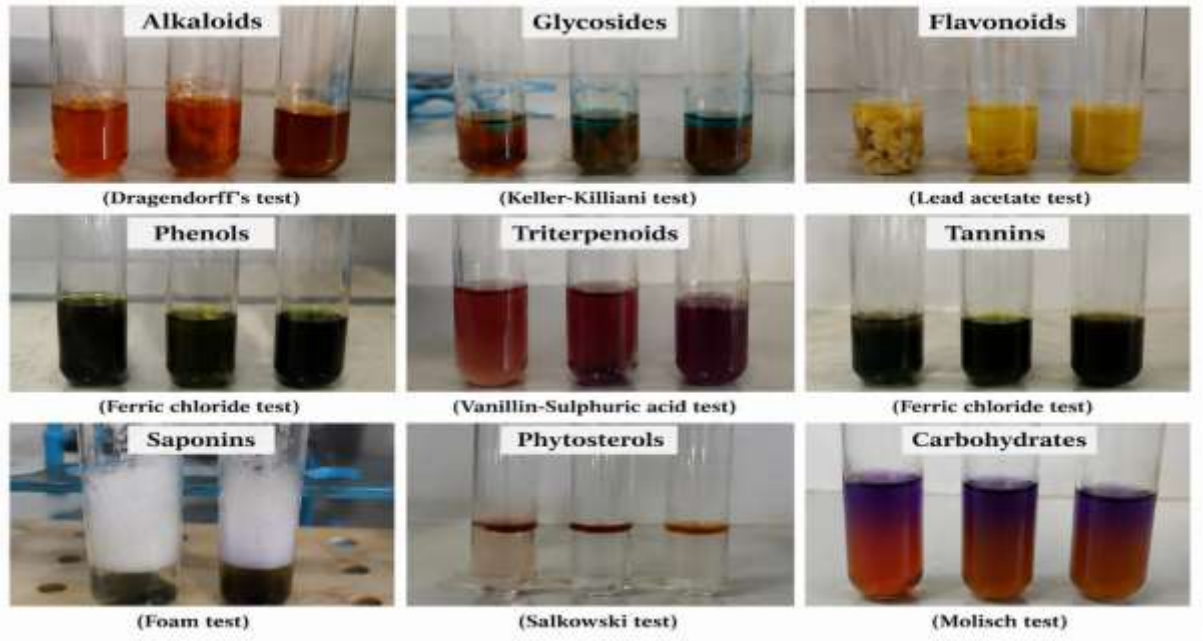
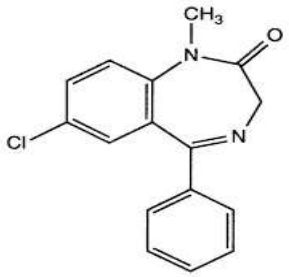
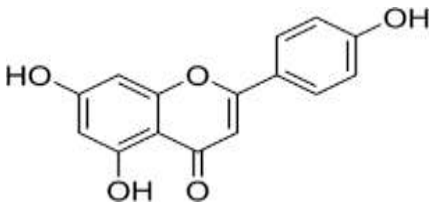
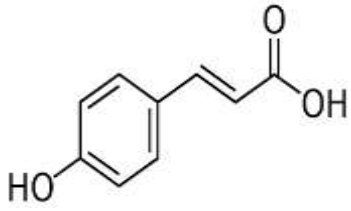
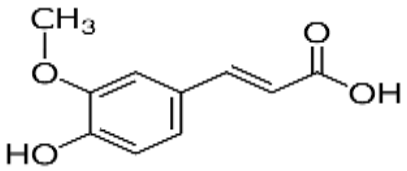
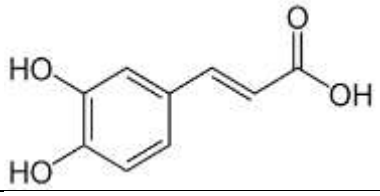
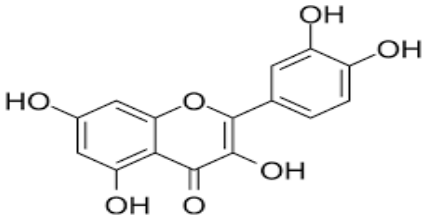
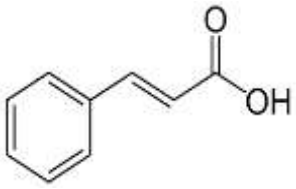


Image No. 5: Phytochemical analysis of Constituents

3.2Molecular Docking:

Table no 2: Molecular docking of the GABA A receptor protein (6D6T) by using software VLife-MDS Version 4.4 to 4.6.

Sr. No.	Compound Name	Structure	Docking Score
1.	Diazepam		-2.474235
2.	Apigenin		-5.073651
3.	P- Coumaric acid		-4.709409

4.	Ferulic acid		-4.630488
5.	Caffeic acid		-4.376463
6.	Qurecetin		-4.332645
7.	Cinnamic Acid		-3.933817

Target structure:
The structure of the target was obtained from the protein data bank (PDB Code 6D6T)

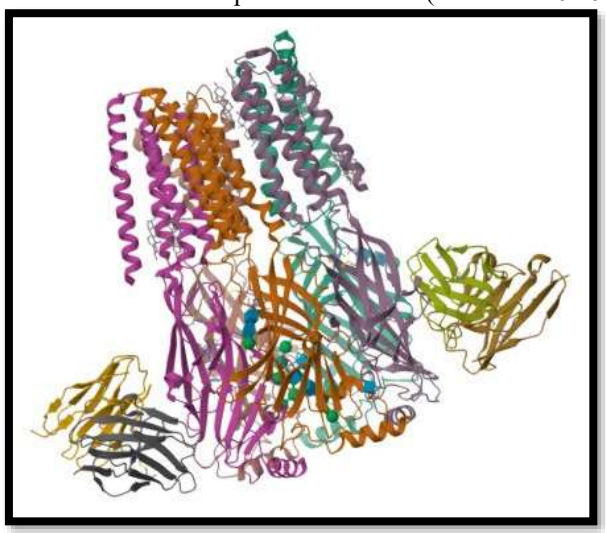


Image No. 6: Target structure PDB Code (6D6T)

Image adopted from (**https://www.rcsb.org/structure/6d6t)**

2. Docking interaction of best – docked molecules:
Docking interactions of compounds with GABA A receptor (protein code 6D6T) Docking interactions of compounds were compared to the standard drug Diazepam. All the selected compounds showed good docking scores and docking interactions. The 2D and 3D interaction of standard drug and selected compound were drawn by using the software Vlife MDS 4.46.

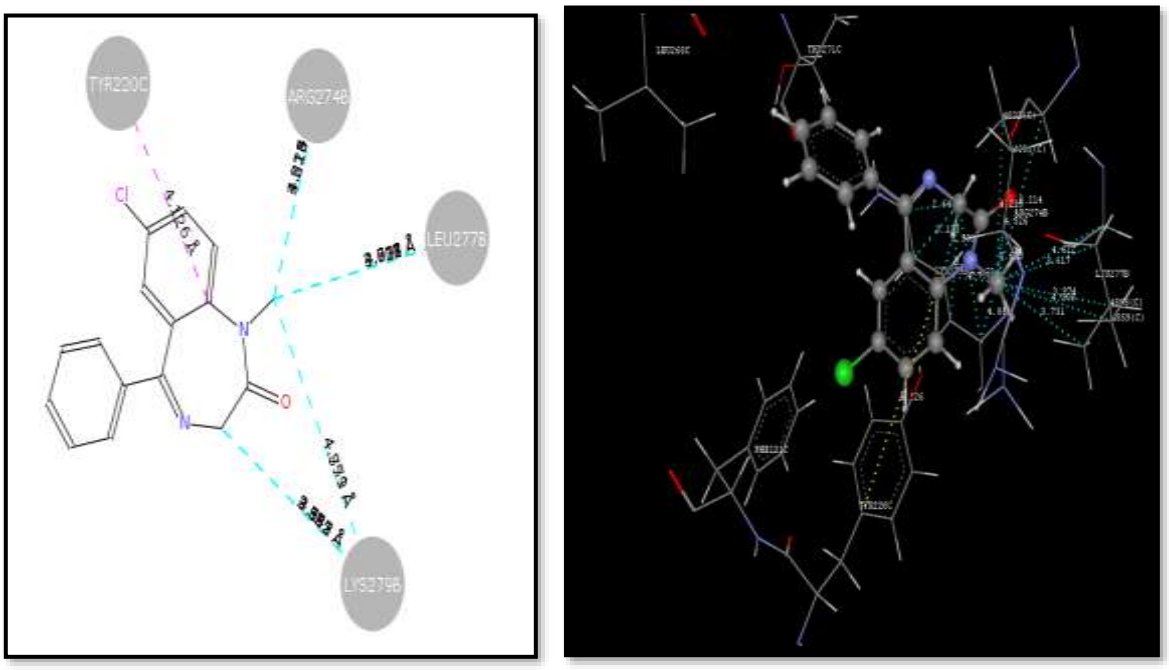


Image No. 7:2D & 3D interaction of Diazepam with GABA A receptor (PDB Code: 6D6T)

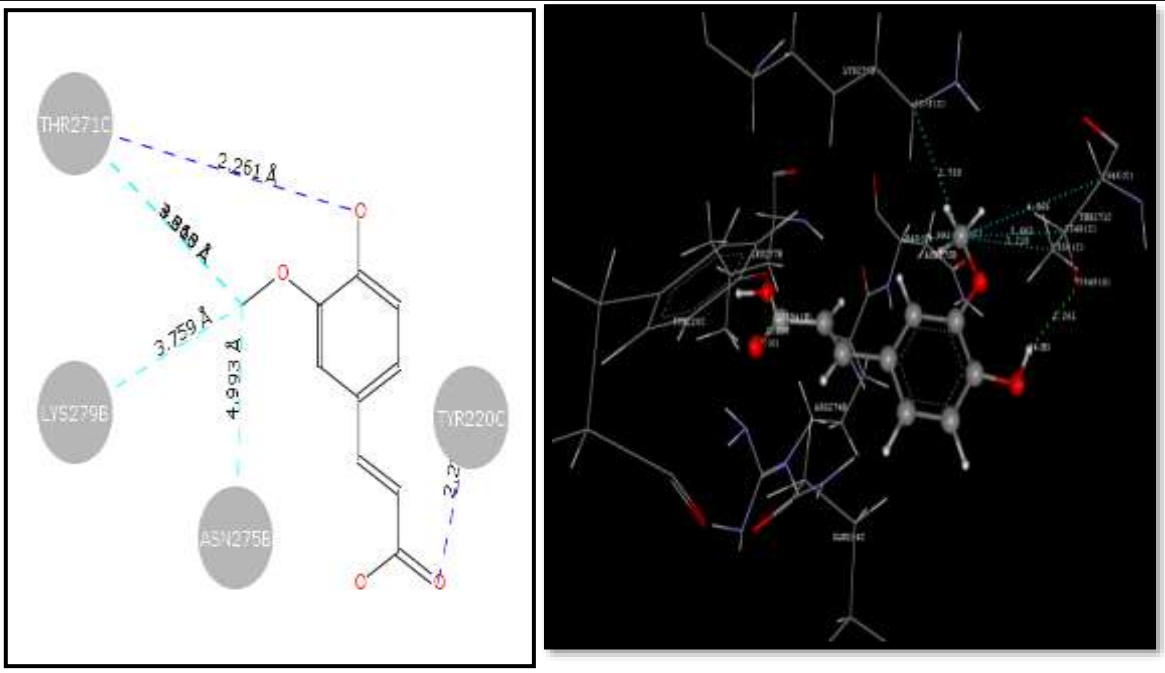


Image No. 8: 2D & 3D interaction of Ferulic acid with GABA A receptor (PDB Code: 6D6T)

3.3In vitro studies:

1.GABA Transaminase (GABA-T) Inhibition Assay:

Table No.3: Effect of *Hamelia patens* Extract on GABA-T Inhibition

Sample	**IC_{50} (µg/mL)**
Hamelia patens Extract	112.6 ± 3.4
Vigabatrin	41.8 ± 1.8

Table No.4: IC_{50} Values

Concentration (µg/mL)	% Inhibition by HP Extract	% Inhibition by Vigabatrin
25	18.6 ± 1.24	32.4 ± 1.15
50	31.8 ± 1.37	48.7 ± 1.28
100	47.5 ± 1.52	65.3 ± 1.44
200	63.2 ± 1.68	81.6 ± 1.56
400	78.4 ± 1.81	92.8 ± 1.32

The methanolic extract of *Hamelia patens* exhibited significant concentration-dependent inhibition of GABA transaminase enzyme activity. At 400 µg/mL, the extract produced 78.4 ± 1.81% inhibition, whereas the standard drug Vigabatrin showed 92.8 ± 1.32% inhibition at the same concentration. The IC_{50} value of the extract was found to be 112.6 ± 3.4 µg/mL, indicating moderate GABA-T inhibitory activity. These findings suggest that *Hamelia patens* may exert anxiolytic effects by increasing brain GABA levels through inhibition of GABA metabolism.

2.DPPH Free Radical Scavenging Assay:

Table No 5: DPPH Radical Scavenging Activity

Concentration (µg/mL)	HP Extract (% Inhibition)	Ascorbic Acid (% Inhibition)
25	22.8 ± 1.16	38.5 ± 1.24
50	36.9 ± 1.28	56.2 ± 1.36
100	52.7 ± 1.42	71.8 ± 1.27
200	68.5 ± 1.54	85.4 ± 1.18
400	84.3 ± 1.37	95.6 ± 1.12

Table No.6: IC_{50} Values

Sample	IC_{50} (µg/mL)
Hamelia patens Extract	94.8 ± 2.8
Ascorbic Acid	34.6 ± 1.5

The *Hamelia patens* extract demonstrated significant DPPH free radical scavenging activity in a concentration-dependent manner. The percentage inhibition increased from 22.8 ± 1.16% at 25 µg/mL to 84.3 ± 1.37% at 400 µg/mL. The sandard antioxidant Ascorbic Acid exhibited higher scavenging activity, producing 95.6 ± 1.12% inhibition at 400 µg/mL. The IC_{50} value of *Hamelia patens* extract was found to be 94.8 ± 2.8 µg/mL, whereas Ascorbic Acid showed an IC_{50} value of 34.6 ± 1.5 µg/mL. These findings indicate that *Hamelia patens* possesses considerable antioxidant activity, which may contribute to its anxiolytic effect by reducing oxidative stress associated with anxiety disorders.

3.4 In Vivo Anxiolytic Activity:

1) Elevated Plus-Maze Test:

Table No.7: Effect of *hamelia patens* extract and diazepam on elevated plus-maze test in mice

Treatment group	Time spent in open arm (Sec)	Time spent in closed arm (Sec)	No. of open arm entry (N)	No. of closed arm entry (N)
Group I Normal control	134.0 ± 2.26	134.0 ± 2.26	134.0 ± 2.26	134.0 ± 2.26
Group II Negative control	104.0 ± 1.66###	104.0 ± 1.66###	104.0 ± 1.66###	104.0 ± 1.66###
Group III Standard	168.0 ± 2.18***	168.0 ± 2.18***	168.0 ± 2.18***	168.0 ± 2.18***
Group IV Test 1 (100mg/kg HP) Extract	118.0 ± 2.04*	118.0 ± 2.04*	118.0 ± 2.04*	118.0 ± 2.04*
Group V Test 2 (200mg/kg HP) Extract	142.0 ± 1.95**	142.0 ± 1.95**	142.0 ± 1.95**	142.0 ± 1.95**
Group VI Test 3 (400mg/kg HP) Extract	158.0 ± 2.11***	158.0 ± 2.11***	158.0 ± 2.11***	158.0 ± 2.11***

**Values are expressed as Mean ± S.E.M. (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 compared with the normal control group. *p < 0.05, **p < 0.01, *p < 0.001 compared with the negative control group using one-way ANOVA followed by Tukey–Kramer multiple comparison test.

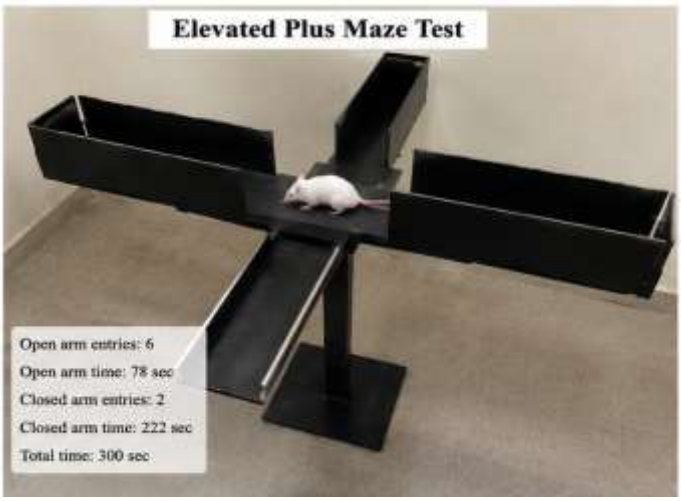


Image No. 9: elevated plus maze apparatus

2) Open Field Test:

Table No. 8: Effect of *Hamelia Patens* and diazepam on an open field test in mice.

Treatment group	No. of square crossed	Rearing	Self-Grooming	Time spent in central squares
Group I Normal control	34.5±1.839	11.6±1.382	12.16±1.138	63.8±3.280
Group II Negative control	24.66± 1.333$^{\#\#}$	5±1.238#	19.66±3.373$^{\#}$	54±3.070$^{\#}$
Group III Standard	55± 1.880***	20.8±1.249***	7.5±0.7638***	106±1.430***
Group IV Test 1 (100mg/kg MLCL) Extract	38.8± 1.973***	11.1±1.641^{ns}	11.16±0.8724**	74.±1.874***
Group V Test 2 (200mg/kg MLCL) Extract	42± 0.894***	12.5±1.607*	9.5±0.4282**	90±0.494***
Group VI Test 3 (400mg/kg MLCL) Extract	47.5± 2.778***	17.3±2.028***	8.5±0.8851***	93.8±1.249***

(Values are in Mean ± S.E.M (n=6); $^{\#}$p<0.05, $^{\#\#}$p<0.01, when compared with normal control. *p<0.05, **p<0.01, ***p<0.001 using One way ANOVA followed by Tukey-Kramer test when compared with negative control.

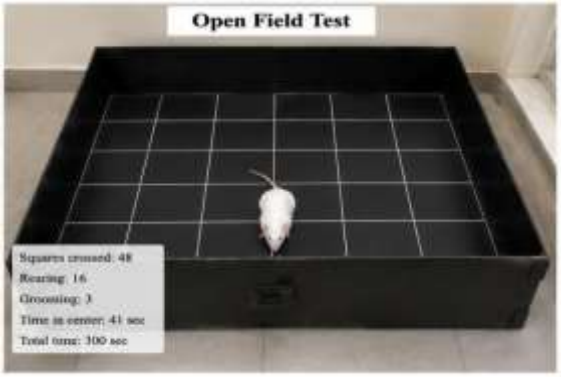


Image No.10: open field test apparatus

3) Y-Maze Test

Table No. 9: Effect of *Hamelia Patens* extract and diazepam on Y-Maze test in mice.

Treatment Group	Total Arm Entries (N)	Spontaneous Alternation (%)	Time Spent in Arms (sec)
Group I Normal Control	24.5 ± 1.02	72.4 ± 1.85	180.2 ± 3.12
Group II Negative Control (Caffeine 100 mg/kg)	18.3 ± 0.95###	48.6 ± 1.42###	142.8 ± 2.65###
Group III Diazepam (4 mg/kg, i.p.)	27.8 ± 1.15***	78.5 ± 1.63***	195.4 ± 2.48***

Group IV HP Extract (100 mg/kg)	20.9 ± 0.88*	57.2 ± 1.74*	156.6 ± 2.91*
Group V HP Extract (200 mg/kg)	23.6 ± 1.06**	66.8 ± 1.58**	171.5 ± 2.37**
Group VI HP Extract (400 mg/kg)	26.1 ± 0.97***	74.3 ± 1.46***	188.2 ± 2.21***

Values are in Mean ± S.E.M (n=6); #p<0.05, ## P<0.01 when compared with normal control. *p<0.05, **p<0.01 ***p<0.001 using One way ANOVA followed by Tukey-Kramer test when compared with negative control

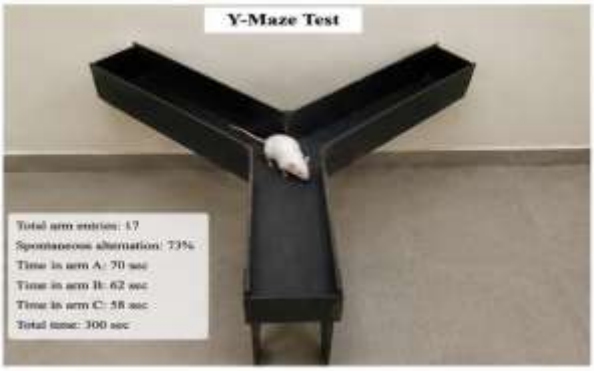


Image No. 11:Ymaze test apparatus

4) T-Maze Test:

Table No. 10: Effect of *Hamelia Patens* and diazepam on T-Maze test in rats

Treatment Group	Total Arm Entries (N)	Correct Arm Choice (%)	Spontaneous Alternation (%)	Time Spent in Goal Arm (sec)
Group I Normal Control	23.8 ± 1.04	74.5 ± 1.62	71.8 ± 1.84	182.4 ± 2.75
Group II Negative Control (Caffeine 100 mg/kg)	17.5 ± 0.89###	48.3 ± 1.45###	46.8 ± 1.63###	136.5 ± 2.42###
Group III Diazepam (4 mg/kg, i.p.)	27.4 ± 1.12***	81.6 ± 1.38***	79.2 ± 1.56***	198.6 ± 2.31***
Group IV HP Extract (100 mg/kg)	20.6 ± 0.95*	58.7 ± 1.71*	56.4 ± 1.82*	154.3 ± 2.68*
Group V HP Extract (200 mg/kg)	23.1 ± 1.08**	68.5 ± 1.52**	65.7 ± 1.48**	171.8 ± 2.54**
Group VI HP Extract (400 mg/kg)	25.8 ± 0.98***	76.9 ± 1.44***	74.5 ± 1.39***	189.2 ± 2.17***

(Values are in Mean ± S.E.M (n=6); #p<0.05 when compared with normal control. *p<0.05, , ***p<0.001 using One way ANOVA followed by Tukey-Kramer test when compared with negative control.

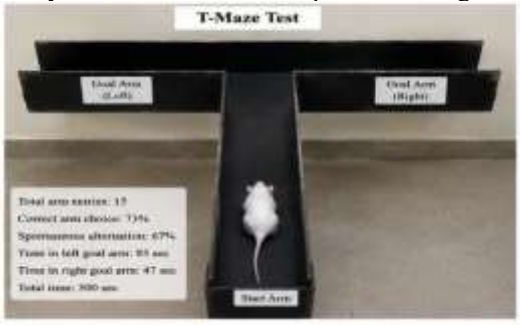


Image No.12: T maze test apparatus

4. Discussion

The present study demonstrated that the methanolic leaf extract of *Hamelia patens* possesses significant anxiolytic activity, as evidenced by phytochemical, molecular docking, in vitro, and in vivo investigations. Preliminary

phytochemical screening confirmed the presence of flavonoids, phenolic compounds, alkaloids, glycosides, tannins, saponins, and triterpenoids, which are known to exhibit neuroprotective and antioxidant properties. Molecular docking revealed strong interactions of major phytoconstituents, particularly apigenin, with the GABA_A receptor, suggesting modulation of GABAergic neurotransmission as a possible mechanism of action.

The extract exhibited concentration-dependent inhibition of GABA-transaminase and significant antioxidant activity in the DPPH assay, indicating its potential to increase endogenous GABA levels while reducing oxidative stress. Behavioural studies further supported these findings, as treatment with *Hamelia patens* significantly improved anxiety-related parameters in the Elevated Plus Maze, Open Field Test, Y-Maze, and T-Maze. The anxiolytic effect was dose dependent, with the 400 mg/kg dose producing responses comparable to diazepam.

Overall, the findings suggest that the anxiolytic activity of *Hamelia patens* may result from the combined effects of GABAergic modulation, GABA-transaminase inhibition, and antioxidant activity. Further studies are required to isolate the active constituents, elucidate their molecular mechanisms, and establish their long-term safety and clinical efficacy.

5. Conclusion

The present study provides scientific evidence that the methanolic leaf extract of *Hamelia patens* possesses significant anxiolytic activity. The extract showed favourable phytochemical composition, strong interactions with the GABA_A receptor, significant GABA-transaminase inhibitory and antioxidant activities, and marked anxiolytic effects in validated behavioural models. Among the tested doses, 400 mg/kg exhibited the greatest efficacy, producing effects comparable to diazepam.

These findings indicate that *Hamelia patens* is a promising natural source of anxiolytic compounds and may serve as a potential candidate for the development of safer plant-based therapies for anxiety disorders. However, further studies on bioactive compound isolation, pharmacokinetics, chronic toxicity, and clinical evaluation are necessary before its therapeutic application can be confirmed.

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