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RESEARCH ARTICLE
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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF *ACHYRANTHES ASPERA L.* STEM EXTRACTS FOR ANTIANXIETY ACTIVITY ON WISTAR RATS

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ABSTRACT:
The present study investigates the phytochemical composition and pharmacological evaluation of *Achyranthes aspera* L. stem extracts for anti-anxiety activity in wistar rats. *Achyranthes aspera*, a well-known medicinal plant, has been traditionally used for the treatment of various ailments, including anxiety. The stem extracts of *A. aspera* were prepared using different solvents and subjected to comprehensive phytochemical screening, revealing the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and glycosides. These compounds are known for their potential therapeutic properties. The anti-anxiety activity was evaluated using established animal models, including the elevated plus maze, open field test, and light/dark box test. The results demonstrated a significant reduction in anxiety-like behavior in rats treated with the stem extract compared to the control group. Furthermore, the pharmacological effects were comparable to that of standard anxiolytic drugs, indicating the potential of *Achyranthes aspera* stem extracts in managing anxiety disorders. The study also suggests a possible mechanism of action,

involving the modulation of neurotransmitter systems, although further investigations are required to elucidate the exact molecular pathways. This research supports the traditional use of *Achyranthes aspera* as an anxiolytic agent and provides a scientific basis for its potential therapeutic application in the management of anxiety disorders.

KEYWORDS: *Achyranthes aspera*, phytochemical screening, anti-anxiety activity, anxiolytic, elevated plus maze, open field test etc.

INTRODUCTION:

Anxiety disorders are a group of debilitating neuropsychiatric conditions characterized by excessive fear, worry, and apprehension that can interfere with daily life. These disorders rank among the most common mental health issues globally, significantly contributing to the global burden of disease. While synthetic medications like benzodiazepines and selective serotonin reuptake inhibitors (SSRIs) are widely used to manage anxiety, their prolonged use often leads to adverse effects such as sedation, dependency, cognitive impairment, and withdrawal symptoms. Consequently, there is a growing demand for safer, effective, and affordable alternatives, particularly those derived from natural sources. Medicinal plants, with their rich repository of bioactive compounds, have emerged as promising candidates for addressing this need.[1-5]

Achyranthes aspera L., commonly known as *Apamarga*, is a medicinal plant belonging to the Amaranthaceae family. Found abundantly in tropical and subtropical regions, this plant has been revered in traditional medicine systems like Ayurveda, Unani, and Siddha for its diverse therapeutic potential. Various parts of the plant, including its stems, roots, leaves, and seeds, have been used in the treatment of ailments ranging from gastrointestinal disorders and respiratory issues to inflammatory conditions and metabolic syndromes. Notably, Achyranthes aspera L. has been recognized for its neuropharmacological properties, yet its anxiolytic potential remains underexplored in modern scientific research.[6-8]

The pharmacological properties of Achyranthes aspera L. are attributed to its rich phytochemical composition, including saponins, alkaloids, flavonoids, and tannins, which are known to exhibit a wide range of biological activities. These bioactive compounds have been shown to modulate key neurotransmitter pathways implicated in anxiety, such as the gamma-aminobutyric acid (GABAergic) and serotonergic systems. However, the precise phytochemical profile of the plant's stem and its potential anxiolytic effects require detailed investigation.

This study aims to bridge the gap by conducting a comprehensive phytochemical investigation of Achyranthes aspera L. stem extracts and evaluating their pharmacological potential in mitigating anxiety using established animal models. Utilizing Wistar rats, the research focuses on behavioral assays, such as the elevated plus maze and open field test, to assess anxiolytic efficacy. By correlating the plant’s phytochemical constituents with its pharmacological effects, this study seeks to validate the traditional use of Achyranthes aspera L. as a natural remedy for anxiety and pave the way for its potential development into a plant-based anxiolytic therapy. The findings are expected to contribute valuable insights into the neuropharmacological benefits of medicinal plants and highlight their significance in the quest for safer and more effective treatments for anxiety disorders.[9-12]

MATERIALS AND METHODS:

MATERIALS:

The study utilized various high-quality chemicals, reagents, and drugs obtained from reputed manufacturers for conducting phytochemical investigations and pharmacological evaluations. The anxiolytic reference drug, diazepam, was purchased from Astron Research Ltd., Ahmedabad. The organic solvents such as petroleum ether, chloroform, ethanol, benzene, ethyl acetate, acetone, methanol, and toluene were procured from Fine Chem Industries, Mumbai. Essential reagents including ferric chloride, sulphuric acid, silica gel G (A.R. grade), DPPH, ascorbic acid, hydrogen

peroxide, quercetin, Folin-Ciocalteu reagent, hexane, and sodium carbonate were sourced from Hi Media Lab, Mumbai.

Standard reference compounds such as gallic acid and phloroglucinol were obtained from Loba Chemie Pvt. Ltd., Mumbai, while other critical reagents like hydrochloric acid, zinc dust, sodium hydroxide, and phenolphthalein were supplied by Qualigens Fine Chemicals, Mumbai. These materials were meticulously chosen to ensure reliability and reproducibility of results in phytochemical analysis and pharmacological testing.[13-15]

Collection, Identification, and Authentication of Plant Material

The fresh stems of *Achyranthes aspera* L. were collected from the local region of Beed, Maharashtra. The plant material was authenticated by Dr. Shrirang S. Bodke, Associate Professor and Head of Botany and Horticulture at Yashwant Mahavidyalaya, Nanded. The authentication process confirmed the identity of *Achyranthes aspera* L., with the specimen being documented under reference number H-7. This authentication ensured the botanical accuracy and reliability of the plant material used in the study.[16]

Processing of Crude Drugs

The fresh stems of plant *Achyranthes aspera* were subjected to shade drying and further crushed to coarse powder passed through mesh no. 14 and stored in air tight container for further use.[17]

Physicochemical Evaluation and Phytochemical Screening

The physicochemical evaluation of *Achyranthes aspera* L. stem included extractive value, ash content, and moisture determination. Extractive values were calculated by macerating 4 g of air-dried powder with solvents like petroleum ether, chloroform, and n-butanol. Total ash, acid-insoluble ash, and water-soluble ash were determined by igniting the powdered drug, treating the residue with dilute HCl or water, and weighing the remains. Loss on drying (LOD) was assessed by drying 2 g of the sample at 105°C until a constant weight was achieved.[18]

Extraction of Plant Material

The extraction of *Achyranthes aspera* L. stem was conducted using solvents selected based on their extractive values, phytochemical compatibility, and supporting literature. Petroleum ether, ethyl acetate, and ethanol were chosen for the study. The Soxhlet extraction method was employed due to its efficiency, heat stability for plant constituents, and ability to ensure complete extraction in less time. This method facilitated optimal solvent penetration and ensured the extraction of desired phytochemicals.

Three extracts-petroleum ether, ethyl acetate, and ethanol were prepared using the continuous hot extraction method with Soxhlet apparatus. For each extraction, 270 g of dried stem powder was processed with 1500 ml of the respective solvent until the siphon tube showed a colorless solution, indicating completion. The extracts were cooled, concentrated using a rotary evaporator, and stored in airtight containers. The dried mass of each extract was weighed, and the percentage yield was calculated using the formula:

Percentage yield=Weight of extract/Weight of powdered drug×100

Phytochemical Screening of Extracts

The extracts obtained through successive Soxhlet extraction were subjected to qualitative analysis to identify various secondary metabolites. These included carbohydrates, proteins, tannins, steroids, flavonoids, alkaloids, and glycosides. Standard phytochemical screening methods were employed to systematically evaluate the presence of these bioactive compounds in each extract, ensuring a comprehensive assessment of their phytochemical composition.[19]

TLC STUDIES

Thin Layer Chromatography (TLC) is a simple and efficient analytical technique used to separate and identify compounds in a mixture. It involves a stationary phase, typically silica gel or alumina, applied as a thin layer on a plate, and a mobile phase (a solvent or solvent mixture) that migrates across the plate via capillary action. Compounds are separated based on their differential interactions with the stationary and mobile phases, resulting in distinct migration distances. TLC plates are prepared by pouring or spraying, followed by air drying to prevent cracks, and are activated by heating at 100–200°C to enhance adsorbent efficiency. The retention factor (Rf), calculated as the ratio of the distance traveled by a compound to the solvent front, is used for identification and varies with factors such as solvent quality, humidity, and sample concentration. TLC is commonly employed for qualitative analysis of plant extracts to detect phytoconstituents like flavonoids, tannins, glycosides, and amino acids. TLC fingerprinting, observed under UV light or after derivatization, provides a characteristic profile of bioactive compounds, aiding in the preliminary evaluation of plant extracts.[20]

QUANTITATIVE ANALYSIS

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extract was evaluated using the Folin–Ciocalteu method, which is based on the oxidation of phenolic groups by phosphomolybdate and phosphotungstate, forming a green-blue complex measurable at 666 nm. Extract solutions (50 µg/mL) were prepared and made up to 3 mL with distilled water. To this, 0.5 mL of Folin–Ciocalteu reagent (diluted 1:10) was added and mixed thoroughly for 3 minutes, followed by the addition of 2 mL of 7% sodium carbonate. The volume was adjusted to 10 mL with distilled water, and the mixture was incubated in the dark for 60 minutes. Absorbance was measured at 666 nm, and TPC was calculated using a gallic acid calibration curve (2–10 µg/mL) with an equation derived from the standard curve (r^2 = 0.9919).[21-23]

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method. This method relies on the nitration of catechol-containing aromatic rings, forming a yellow complex with $AlCl_3$ that turns red upon the addition of NaOH. A 1 mL extract solution (50 µg/mL) was mixed with 4 mL distilled water, followed by 0.3 mL of 5% sodium nitrite. After 5 minutes, 0.3 mL of 10% aluminum chloride was added, followed by 2 mL of 1 M sodium hydroxide after another 5 minutes. The mixture was adjusted to 10 mL with distilled water, incubated for 30 minutes, and the absorbance was recorded at 510 nm. Quercetin was used as the standard, and TFC was calculated using a quercetin calibration curve (2–10 µg/mL) with an equation derived from the standard graph (r^2 = 0.9911). Results were expressed as milligrams of quercetin equivalent (mg QE) per gram of dry weight.[24-26]

In-vitro Anti-oxidant studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay evaluates antioxidant activity by measuring the reduction of the purple-colored DPPH radical, resulting in a decrease in absorbance at 517 nm. Extracts (ethyl acetate and ethanol) and quercetin (standard) were dissolved in methanol, and various concentrations (20–100 µg/mL) were prepared. Each sample was mixed with 0.4 mL of 0.1 mM DPPH solution, and the volume was adjusted to 10 mL with methanol. A control without extract was also prepared. After vigorous shaking and incubation in the dark for 1 hour at room temperature, absorbance was measured at 517 nm using a UV-visible spectrophotometer. The radical scavenging activity was expressed as a percentage reduction of DPPH radicals, with EC_{50} values calculated for comparison.[27-31]

Acute toxicity studies

Acute toxicity studies are essential for determining the safe dose of a new chemical substance by assessing dose-related responses and potential side effects. Acute oral toxicity testing is a preliminary step in evaluating the toxic characteristics of drug compounds, often determining the lethal dose (LD_{50}) that causes death in 50% of test animals. The OECD provides ethical guidelines for such studies, aiming to minimize animal use and suffering. Key protocols include Guideline 420 (fixed dose), Guideline 423 (acute toxic class), and Guideline 425 (up-and-down method). For the current study, the acute oral toxicity of *Achyranthes aspera* L. extracts was assessed following OECD Guideline 423, enabling classification of the test substance into a toxicity category based on fixed LD_{50} cut-off values.[32-34]

Selection and procurement of animals

The study was conducted following the approval of the Institutional Animal Ethics Committee (IAEC) and in compliance with CPCSEA guidelines. Male and female Wistar rats, each weighing 200–250 g, were selected for the experiment, which utilized the elevated plus maze model. A total of 84 rats were used in the study. The standard drug was administered intravenously, while the test compound was given orally.[35-37]

EVALUATION OF ANTIANXIETY ACTIVITY

Elevated plus maze test

The elevated plus maze test was used to assess the anxiolytic effects of different treatments. The maze consists of two open arms (35 × 15 × 15 cm) and two enclosed arms of the same size, arranged so that the open arms are opposite each other. The maze is elevated 50 cm above the ground, with each arm measuring 10 cm in width. Wistar rats (200–250 g) were housed in pairs for 10 days prior to the test, during which they were handled every other day to reduce stress. The animals were divided into six groups, each consisting of six rats: Control, Standard drug-treated, and four groups treated with varying doses of plant extracts (higher and lower). Thirty minutes after oral or intraperitoneal administration of the test and standard drugs, respectively, each rat was placed at the center of the maze, facing one of the enclosed arms.[39-40] The procedure was recorded using a video camera mounted directly above the maze. The groups were as follows:

1. Group A - Control (CMC 0.5%)
2. Group B - Standard (Diazepam 2 mg/kg)
3. Group C - Test Dose 1 (AAAE 100 mg/kg)
4. Group D - Test Dose 2 (AAAE 200 mg/kg)
5. Group E - Test Dose 1 (AAETH 100 mg/kg)
6. Group F - Test Dose 2 (AAETH 200 mg/kg).

Evaluation parameters included the total number of entries into the open and enclosed arms, the time spent in each arm, and the time spent examining the open arms.[41-42]

RESULTS AND DISCUSSIONS:

Physical Evaluation of plant material

The table 1 presents physical evaluation results of the plant material. The Loss on Drying (LOD) was 18.23%, indicating moisture content. The Total Ash Value was 9%, with Acid Insoluble Ash and Water Soluble Ash at 2.5% and 5.5%, respectively, reflecting the inorganic residue and solubility characteristics. The Extractive Values showed that Water had the highest yield at 39%, followed by Ethyl Acetate (32%) and Ethanol (31.5%), while Petroleum Ether (18.5%) and Acetone (10.5%) were less efficient. These results guide the selection of solvents for further phytochemical analysis.

Table 1: Physiochemical Parameters

Sr. No.	Parameter	Value
1	Loss on Drying (LOD)	18.23%
2	Total Ash Value	9%
3	Acid Insoluble Ash	2.5%
4	Water Soluble Ash	5.5%
5	Petroleum Ether Extract	18.5%
6	Acetone Extract	10.5%
7	Ethyl Acetate Extract	32%
8	Chloroform Extract	22.5%
9	Methanol Extract	11%
10	Ethanol Extract	31.5%
11	Water Extract	39%

% Yield of AA Extract

The extraction results highlight the influence of solvents on yield. Using 270 g of the drug and 1500 ml of solvent, Ethanol yielded the highest extract (15.19 g, 5.84%), followed by Ethyl Acetate (11.01 g, 4.23%). Petroleum Ether produced the least extract (2.80 g, 1.07%). All extracts exhibited a sticky consistency with a dark brown color. These results suggest ethanol as the most effective solvent for extracting bioactive components from the plant material.

Table 2: Appearance and % Yield of AA Extract

Sr. No.	Drug Taken (g)	Solvent Used (ml)	Consistency	Color of Extract	Yield (g)	% Yield
1	270	1500 (Petroleum Ether)	Sticky	Dark Brown	2.80	1.07%
2	270	1500 (Ethyl Acetate)	Sticky	Dark Brown	11.01	4.23%
3	270	1500 (Ethanol)	Sticky	Dark Brown	15.19	5.84%

Preliminary Phytochemical evaluation

The chemical test conducted on plant extracts using different solvents (Petroleum Ether, Ethyl Acetate, and Ethanol) revealed distinct phytochemical profiles. Carbohydrates were absent in Petroleum Ether extracts but present in Ethyl Acetate and Ethanol, as indicated by positive results in Molish and Fehling's tests. Proteins were detected in Ethyl Acetate and Ethanol but not in Petroleum Ether, while Millions test was positive only for Petroleum Ether. Amino acids were absent across all extracts. Steroids were identified in Petroleum Ether and Ethanol extracts but not in Ethyl Acetate, as shown by Salkowski and Libermann-Burchard tests.

Glycosides were not tested conclusively, while Flavonoids were prominent in Ethyl Acetate and Ethanol extracts with positive Shinoda, Lead Acetate, and Sulphuric Acid tests. Tannins and phenolic compounds were detected in Ethyl Acetate and Ethanol using Lead Acetate and Ferric Chloride tests, but Nitric Acid tests were negative for all solvents. Alkaloids were observed in Ethyl Acetate and Ethanol extracts through Mayer's and Hager's tests, while Wagner's test was negative in all cases. These findings demonstrate Ethanol as the most effective solvent for extracting a wide range of phytochemicals.

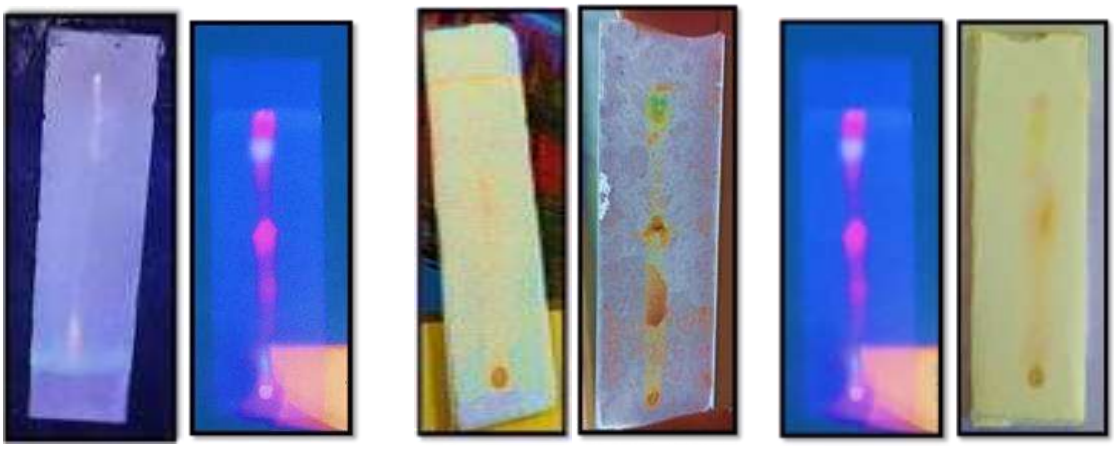
Table 3: Preliminary Phytochemical evaluation

Chemical test	Pet. ether	Ethyl acetate	Ethanol
Test for carbohydrates			
1)Molish test	-	+	+
2)Fehling's test	-	+	+
3)Benedict's test	-	-	-
Test for protein			
1)Biuret test	-	+	+
2)Millions test	+	-	-
Test for amino acids			

1)Ninhydrin test	-	-	-
Test for steroids			
1)Salkowski test	-	+	+
2)Libermann-Burchard test	+	-	-
Test for glycosides			
1)Foam test			
Test for flavonoids			
1)Shinoda test	-	+	+
2)Lead acetate test	+	+	+
3)Sulphuric acid test	-	-	+
Test for tannins and phenolic compound			
1)Lead acetate test	+	+	+
2)Dil. Nitric acid test	-	-	-
3)Ferric chloride test	-	+	+
Test for alkaloids			
1)Mayer's test	-	+	+
2)Hager's test	-	+	+
3)Wagner's test	-	-	-

Thin Layer Chromatography

The Thin Layer Chromatography (TLC) study revealed the presence of diverse phytochemicals in Petroleum Ether, Ethyl Acetate, and Ethanol extracts using different solvent systems and spraying agents. The Petroleum Ether extract showed Rf values of 0.87 (brown, flavone glycosides) and 0.52 (yellow, rutin). The Ethyl Acetate extract demonstrated a broader chemical profile with Rf values of 0.85 (blue, chlorogenic acid), 0.55 (green, flavonoid), 0.45 (yellow, rutin), 0.20 (brown, flavone glycosides), and 0.18 (pink, gallic acid). The Ethanol extract highlighted Rf values of 0.66 (brown, flavone glycosides), 0.49 (green, flavonoid), and 0.38 (pink, gallic acid). The TLC study confirms the presence of key phytochemical constituents such as flavonoids, glycosides, and phenolic acids, with Ethyl Acetate extract showing the highest chemical diversity.



A B C

Figure 1: TLC of AA Extract A) Pet. Ether B) Ethyl Acetate C) Ethanol

Phytochemical Quantitative analysis of Extracts

Total Phenolic Content

The Total Phenolic Content (TPC) analysis of *Achyranthes aspera* stem extracts indicated that ethanol extract (108 mg GAE/g DW) exhibited higher phenolic content compared to the ethyl acetate extract (93.7 mg GAE/g DW). These values were determined using gallic acid as the standard, with results expressed as mean ± SEM (n=3). The data highlights ethanol as the superior solvent for extracting phenolic compounds from the stem of *Achyranthes aspera*.

Table 4: TPC of AA Stem extract

Sr. No	Extracts	Concentration (µg/ml)	Absorbance	TPC (mg/GAE/g)
1	Ethyl acetate	50	0.176±0.002	93.7
2	Ethanol	50	0.202±0.004	108

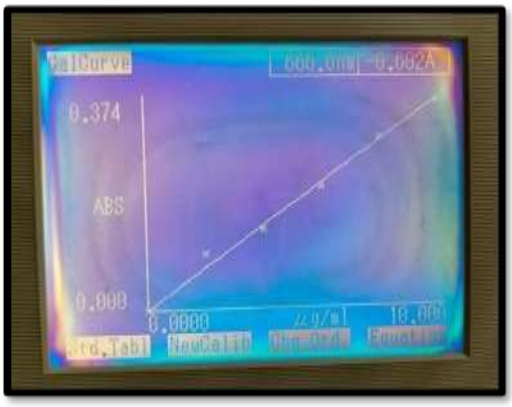


Figure 2: Calibration curve of Gallic Acid

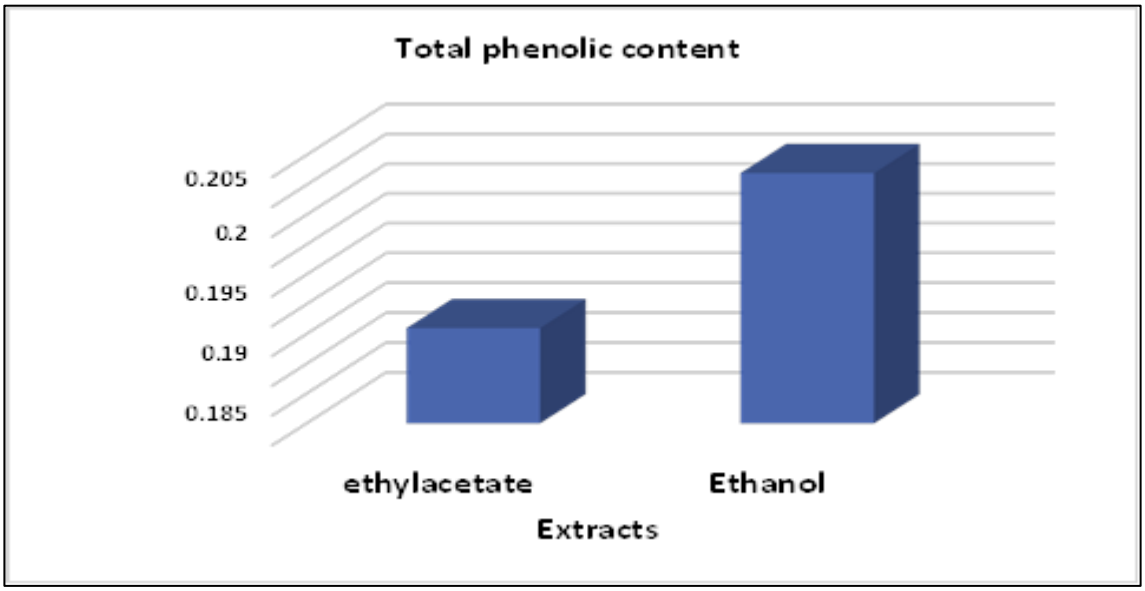


Figure 3: TPC of *Achyranthes aspera* L. Stem extracts

Total Flavonoid Content:

The total flavonoid content (TFC) analysis of *Achyranthes aspera* stem extracts revealed that the Ethyl Acetate extract contains 70.4 mg QE/g DW, while the Ethanol extract has a significantly higher flavonoid content of 160.2 mg QE/g DW. This indicates that the Ethanol extract is richer in flavonoids compared to the Ethyl Acetate extract, highlighting its potential for bioactive

applications. (*Note: mg QE/g DW refers to milligrams of Quercetin Equivalent per gram of dry weight.*)

Table 5: TFC of AA Stem extract

Sr.No.	Extracts	Concentration (µg/ml)	Absorbance	TFC (mg/QE/g)
1	Ethyl acetate	50	0.0032±0.0028	70.4
2	Ethanol	50	0.072±0.0018	160.2

Figure 4: Calibration curve and equation of Quercetin

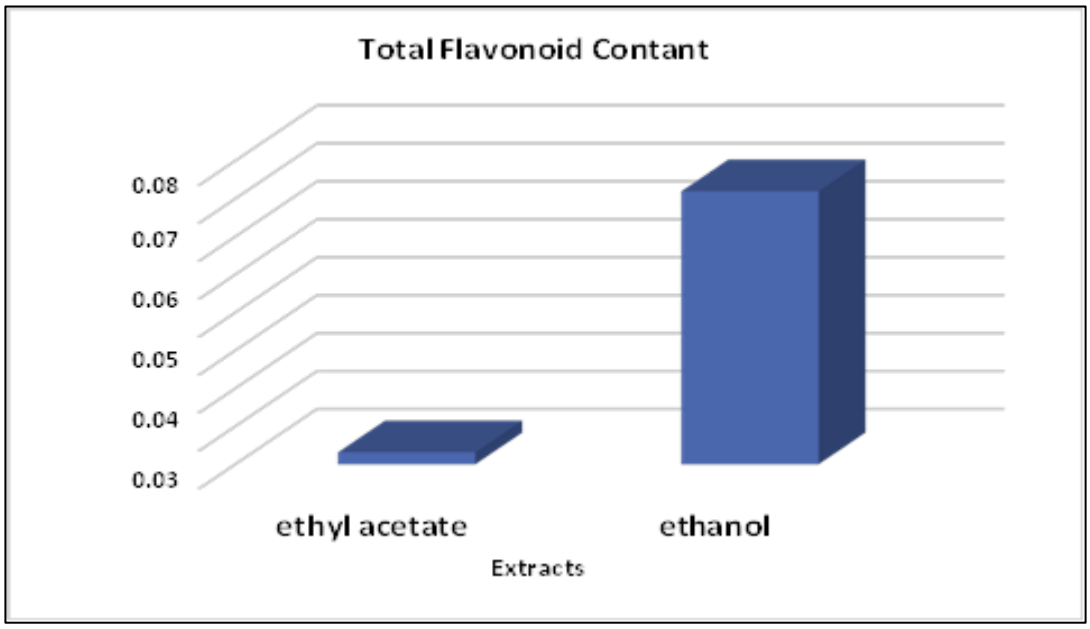


Figure 5: *Achyranthes aspera* L. stem Extracts

Pharmacological screening of plant extracts

In-vitro antioxidant activity

The table 6 presents the percentage inhibition of Gallic acid, Rutin, and Quercetin at different concentrations (20 to 100 µg/ml). It shows that as the concentration increases, the % inhibition for all three compounds also increases, indicating a dose-dependent antioxidant activity. At 100 µg/ml, Quercetin exhibits the highest % inhibition (88.92 ± 0.10), followed by Rutin (81.68 ± 0.13) and Gallic acid (76.14 ± 0.24). These results suggest that Quercetin is the most potent antioxidant among the three compounds tested, with Rutin and Gallic acid showing substantial activity as well.

Table 6: % Inhibition of Gallic acid, Rutin, Quercetin.

Sr.no.	Conc. µg/ml	% inhibition		
		Gallic acid	Rutin	Quercetin
1	20	48.30 ± 0.36	69.26 ± 0.28	44.4 ± 0.10
2	40	53.59 ± 0.21	73.33 ± 0.17	54.20 ± 0.18
3	60	61.37 ± 0.28	76.43 ± 0.18	65.56 ± 0.18
4	80	64.95 ± 0.18	80.09 ± 0.25	76.22 ± 0.17
5	100	76.14 ± 0.24	81.68 ± 0.13	88.92 ± 0.10

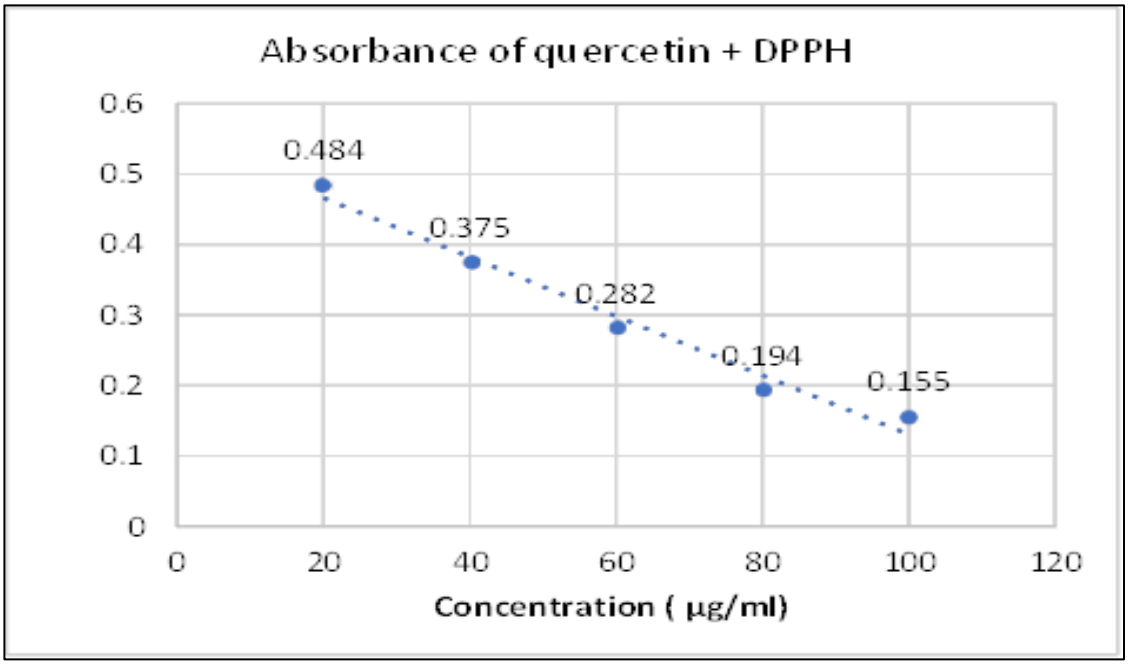


Figure 6:% inhibition of standard compound (antioxidants

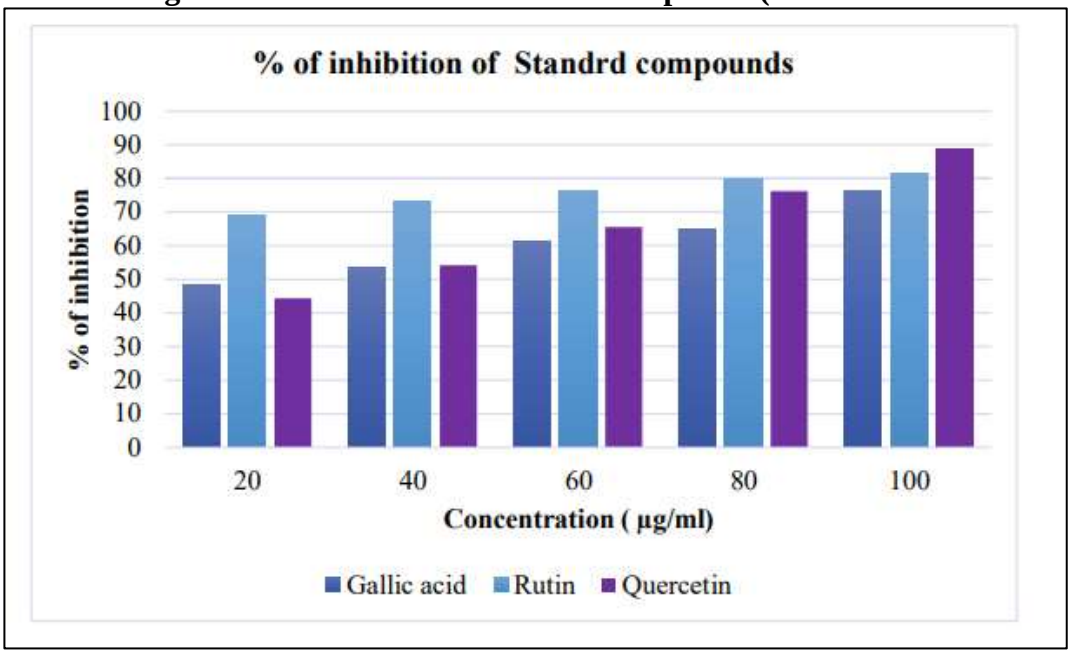


Figure 7: % inhibition of standard compound

The table 7 compares the % inhibition of AA stem extracts (ethyl acetate and ethanol) and Quercetin (as a standard) at varying concentrations (20 to 100 µg/ml). Results reveal a dose-dependent increase in % inhibition across all samples. At lower concentrations (20 µg/ml), the ethanol extract (57.36 ± 0.17) shows higher % inhibition than ethyl acetate (51.24 ± 0.21) and Quercetin (44.4 ± 0.10). This trend continues at higher concentrations, with the ethanol extract demonstrating the highest activity, reaching 86.39 ± 0.22 at 100 µg/ml. Ethyl acetate extract follows with 80.72 ± 0.29, while Quercetin achieves 88.92 ± 0.10. The ethanol extract exhibits superior antioxidant activity, closely matching Quercetin, while the ethyl acetate extract shows moderate activity.

Table 7: % Inhibition of AA stem extract of different concentration.

Sr.No	Conc. (ug/ml)	% inhibition of extract and standard		
		Quercetin	Ethyl Acetate	Ethanol
1	20	44.4 ± 0.10	51.24±0.21	57.36±0.17
2	40	54.20 ± 0.18	60.09±0.12	65.07±0.12
3	60	65.56 ± 0.18	65.53±0.31	70.29±0.14
4	80	76.22 ± 0.17	72.56±0.22	78.68±0.19
5	100	88.92 ± 0.10	80.72±0.29	86.39±0.22

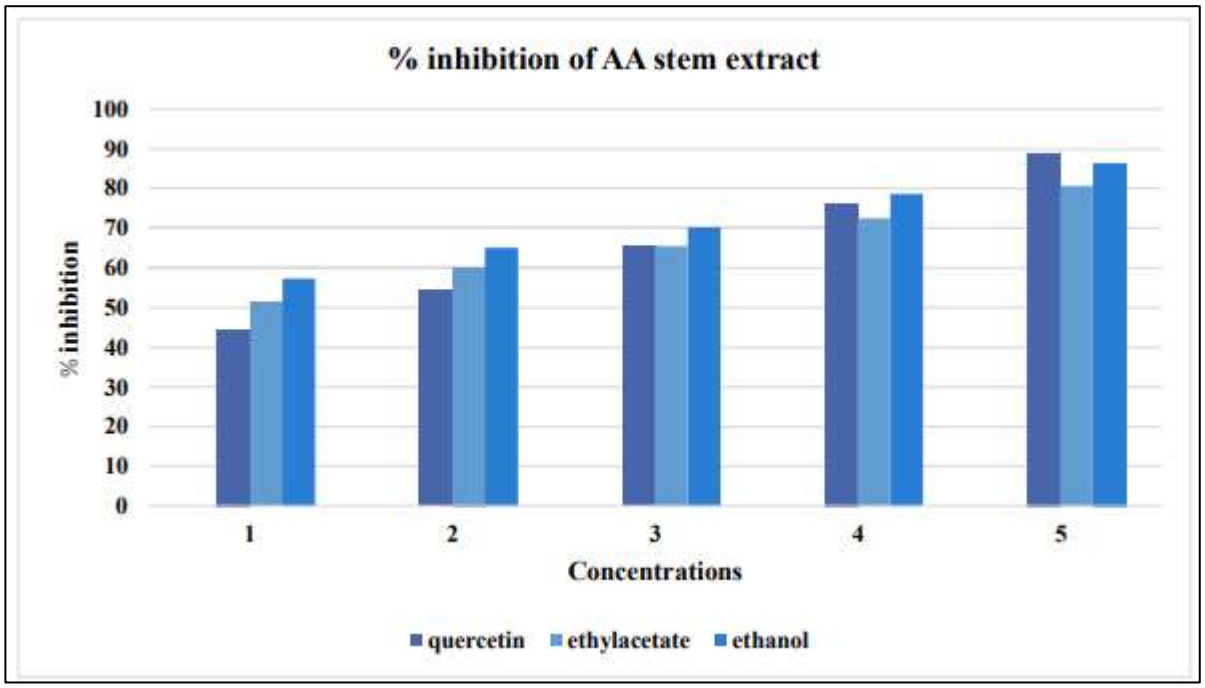


Figure 8: % of inhibition of AA stem extracts

Using the DPPH scavenging assay, the % inhibition of ethyl acetate and ethanolic stem extracts of *Achyranthes aspera* L. was evaluated at 513 nm across concentrations of 20, 40, 60, 80, and 100 µg/ml. The results were compared with Quercetin, used as the reference standard. Both extracts demonstrated significant % inhibition comparable to the standard. Notably, the ethanol extract exhibited the highest % inhibition activity, reaching 86.39% at a concentration of 100 µg/ml, highlighting its superior antioxidant potential.

In-vivo Pharmacological Evaluation of *Achyranthes aspera* Linn.

Acute toxicity studies

Acute toxicity studies of *Achyranthes aspera* Linn. at doses up to 2000 mg/kg revealed no significant behavioral changes or mortality in animals. The results indicated that the median lethal dose (LD50) is likely greater than 2000 mg/kg in rats or mice. Based on this, the safe experimental dose was considered ≤ 2000 mg/kg. Following OECD (423) guidelines, 1/10th of the LD50 was used to determine the maximum safe dose, with selected doses being 100 mg/kg for the lower dose and 200 mg/kg for the higher dose.

In- Vivo Anti- anxiety activity

In the Elevated Plus Maze test, the vehicle-treated rats spent significantly more time in the enclosed arm (173 ± 2.36) and less time in the open arm (110 ± 6.54). They made fewer entries into the open arm (2.5 ± 0.22) and more into the enclosed arm (5.66 ± 0.21). However, both AAEAE (200 mg/kg) and AAETH (200 mg/kg) showed a significant reduction in the time spent in the enclosed arm, indicating a reduction in anxiety. Treatment with AAEAE (200 mg/kg), AAETH (100 mg/kg), AAETH (200 mg/kg), and diazepam (2 mg/kg) resulted in a significant ($p < 0.001$) increase in the time spent in the open arm, suggesting an antianxiety effect. Among these, AAEAE (200 mg/kg) and AAETH (200 mg/kg) exhibited the most prominent antianxiety activity compared to the lower doses of AAEAE (100 mg/kg) and AAETH (100 mg/kg).

Table 8: *Achyranthes aspera* stem extracts mean reading from Elevated plus maze model

Treatment	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	Number of entries in open arm	Number of entries in enclosed arm
Control	110 ± 6.54	173 ± 2.36	2.5 ± 0.22	5.66 ± 0.21
Diazepam (2mg/kg)	240.16 ±1.66**	68.5 ± 1.10**	32.33 ± 0.78**	7.8 ± 0.46**
AAEAE (100mg/kg)	138 ± 2.88**	146.68 ±2.38**	18.16 ± 0.80**	4.16 ± 0.45**
AAEAE (200mg/kg)	198.12 ±2.87**	82.31 ± 2.86**	20.5 ± 0.80**	4.66 ± 0.32**
AAETH (100mg/kg)	148.16 ±1.40**	142.2 ± 1.54**	14.5 ± 0.40**	6.40 ± 0.32**
AAETH (200mg/kg)	210.66 ±1.32**≠	72.81 ± 1.72**≠	22.33 ± 0.82**≠	8 ± 0.38**≠

Each value represents the mean ± S. E. M (n=6)

*Significant difference when standard and test compound with control ($P < 0.05$);

**Highly significant difference when test compared with control ($p < 0.001$); ≠ No significant difference when test compared with standard.

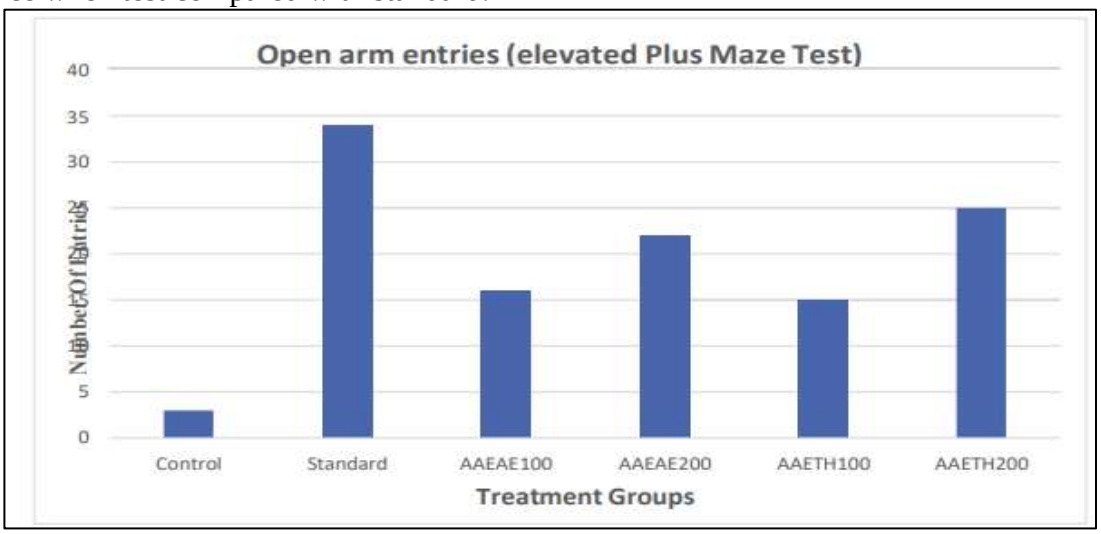


Figure 9: Total number of entries in open arm in elevated plus maze test.

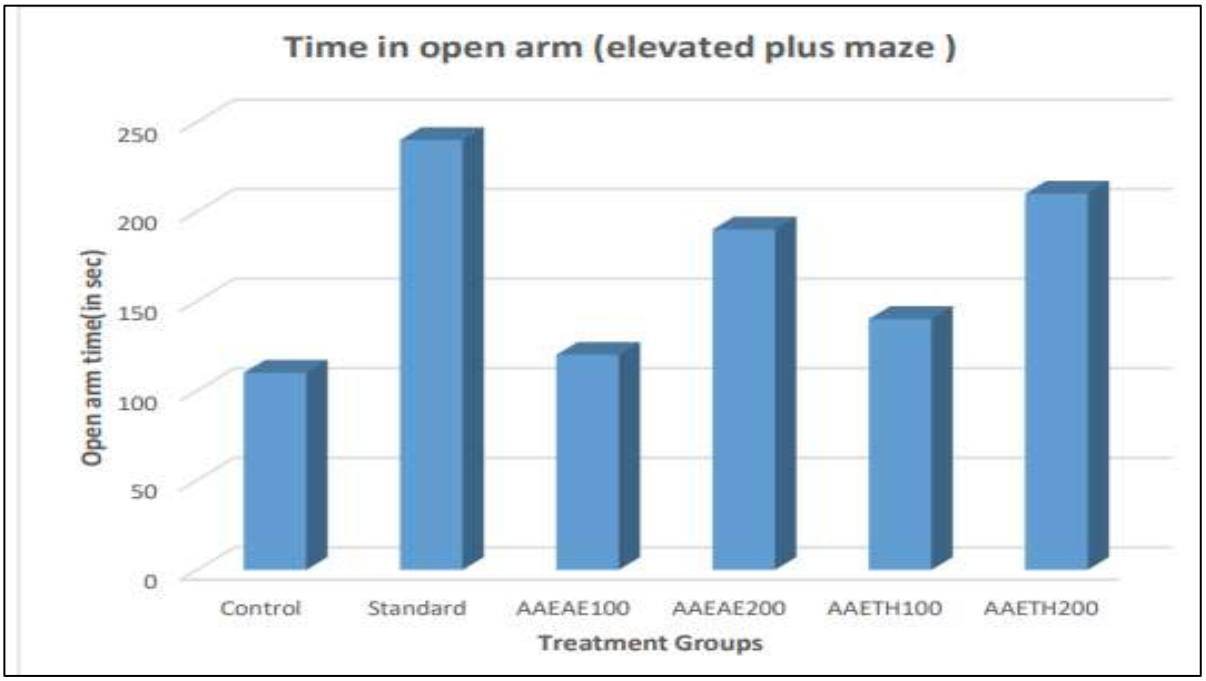


Figure 10: Time Spent in open arm (in sec) in elevated plus maze tes

CONCLUSIONS:

The increasing reliance on medications with side effects has spurred a growing interest in natural remedies. Achyranthes aspera Linn. a plant from the Amaranthaceae family, has not been extensively studied for its anti-anxiety properties. This study aimed to investigate the anti-anxiety activity of its stem extracts. The ethyl acetate, ethanol, and petroleum ether extracts were prepared and analyzed for their phytochemical content, revealing the presence of flavonoids, carbohydrates, alkaloids, and other bioactive compounds.

Ethanol extract showed the highest levels of phenolic and flavonoid compounds, which contributed to its significant antioxidant and anti-anxiety activities. Acute toxicity studies showed no adverse effects up to a dose of 2000 mg/kg, indicating the extracts' safety.

The anti-anxiety effects of the ethyl acetate and ethanol extracts were evaluated using the elevated plus maze model, with both extracts at 200 mg/kg showing results comparable to diazepam, the standard anxiolytic drug. These findings suggest that Achyranthes aspera has potential as a natural anxiolytic agent, warranting further investigation.

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CONFLICTS OF INTERESTS:

All authors have declared no conflict of interest.

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