



ANXIOLYTIC POTENTIAL OF GMELINA ARBOREA EXTRACT USING ELEVATED PLUS MAZE AND LIGHT -DARK MODELS IN EXPERIMENTAL ANIMALS

Shivani S. Jadhav^{1*}, Karan B. More²

¹M. Pharm Student Department of Pharmacology Dr. Vedprakash Patil Pharmacy College, Georai Tanda, Paithan Road, Chh Sambhajinagar.

²Professor, Department of Pharmacology Dr. Vedprakash Patil Pharmacy College, Georai Tanda, Paithan Road, Chh Sambhajinagar.

Article Received on 23 April 2026,
Article Revised on 13 May 2026,
Article Published on 01 June 2026

<https://doi.org/10.5281/zenodo.20441421>

*Corresponding Author

Shivani S. Jadhav

M. Pharm Student Department of Pharmacology Dr. Vedprakash Patil Pharmacy College, Georai Tanda, Paithan Road, Chh Sambhajinagar.



How To Cite This Article: Shivani S. Jadhav^{1*}, Karan B. More². (2026). Anxiolytic Potential of *Gmelina Arborea* Extract Using Elevated Plus Maze and Light- Dark Models in Experimental Animals. World Journal of Pharmacy and Pharmaceutical Sciences, 15(6), 440–470. This work is licensed under Creative Commons Attribution 4.0 International license.

1. ABSTRACT

Anxiety disorders are among the most prevalent neurological conditions worldwide and significantly affect the quality of life of individuals. Although several synthetic anxiolytic drugs are available for clinical use, their long-term administration is often associated with adverse effects such as sedation, tolerance, dependence, and cognitive impairment. Consequently, there is a growing interest in identifying safer and effective alternatives from medicinal plants. The present study was undertaken to evaluate the anxiolytic potential of the ethanolic extract of *Gmelina arborea* leaves using experimental animal models. The collected plant material was authenticated and subjected to morphological, organoleptic, and physicochemical evaluation to ensure its quality and purity. The dried plant material was extracted using ethanol through Soxhlet extraction, and the obtained extract was subjected to preliminary phytochemical

screening. The phytochemical analysis revealed the presence of important bioactive constituents such as flavonoids, phenolic compounds, alkaloids, tannins, glycosides, terpenoids, and saponins. Quantitative analysis confirmed the presence of significant levels of phenolic and flavonoid compounds, which are known to possess antioxidant and neuroprotective properties. An acute toxicity study performed according to OECD Guideline

423 indicated that the extract was safe up to a dose of 2000 mg/kg body weight. Based on the safety profile, two dose levels (100 mg/kg and 200 mg/kg) were selected for pharmacological evaluation. The anxiolytic activity of the extract was assessed using the Elevated Plus Maze and Light–Dark Box behavioral models. The results demonstrated a significant increase in the time spent in open arms and illuminated compartments along with an increase in exploratory behavior compared to the control group. The observed effects were dose-dependent and comparable with the standard drug diazepam. The findings of the present study suggest that *Gmelina arborea* possesses significant anxiolytic activity and may serve as a potential natural therapeutic agent for the management of anxiety disorders.

KEYWORDS: *Gmelina arborea*, Anxiety, Anxiolytic activity, Elevated Plus Maze, Light–Dark Box test, Medicinal plants, Phytochemical screening, Experimental animals.

2. INTRODUCTION

1.1 General introduction to medicinal plants^[1-10]

Medicinal plants have been an essential component of human healthcare systems since ancient times. Across different civilizations, plants have served as a primary source of remedies for the prevention and treatment of numerous diseases. Traditional medical systems such as Ayurveda, Traditional Chinese Medicine, Unani, and other indigenous healing practices have relied extensively on plant-based preparations due to their therapeutic effectiveness and natural origin. Medicinal plants contain a wide variety of biologically active constituents that contribute to their pharmacological effects, making them valuable resources for both traditional healing and modern pharmaceutical development.

1.1.1 Importance of medicinal plants in traditional and modern medicine

Medicinal plants occupy a central role in traditional healthcare systems because of their accessibility, affordability, and therapeutic value. In many parts of the world, especially in developing countries, plant-based medicines remain the primary form of treatment for common ailments. Traditional healers and practitioners have long utilized different plant parts such as leaves, roots, bark, fruits, and seeds for preparing herbal formulations to manage diseases ranging from minor infections to chronic disorders. These plant-based therapies are often administered in the form of decoctions, infusions, powders, and extracts.

In modern medicine, medicinal plants continue to provide significant contributions to therapeutic advancements. Many modern pharmaceutical drugs have originated from natural

sources, particularly plants. Scientific research has validated the therapeutic potential of several plant-derived compounds, leading to their development into standardized medications. Compounds such as morphine, quinine, and paclitaxel are notable examples of drugs that were initially discovered from plants and later developed into widely used pharmaceutical agents. The integration of traditional knowledge with modern scientific techniques has therefore strengthened the role of medicinal plants in contemporary healthcare systems.

1.1.2 Role of plant-derived compounds in drug discovery

Natural products derived from plants have played a crucial role in the discovery and development of new therapeutic agents. Plants produce a diverse array of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, glycosides, and saponins. These phytochemicals often exhibit significant biological activities that can be exploited for therapeutic purposes. Due to their structural diversity and biological specificity, plant-derived compounds have served as valuable lead molecules in the development of numerous pharmaceutical drugs.

Drug discovery from natural sources typically involves several stages, including extraction, isolation, characterization, and pharmacological evaluation of bioactive compounds. Advances in analytical techniques, such as chromatography and spectroscopic methods, have facilitated the identification of new plant-derived compounds with potential medicinal applications. Furthermore, many plant-based molecules provide unique chemical frameworks that are difficult to synthesize artificially, making them indispensable in the development of innovative therapeutic agents. As a result, natural products research continues to be a vital area of pharmaceutical and biomedical investigations.

1.1.3 Advantages of herbal medicines compared with synthetic drugs^[11-14]

Herbal medicines offer several advantages when compared with conventional synthetic drugs. One of the primary benefits is their natural origin, which often contributes to better biocompatibility and reduced toxicity. Many herbal preparations contain a combination of phytochemicals that work synergistically to produce therapeutic effects while minimizing adverse reactions. This multi-component nature can enhance the overall efficacy of herbal formulations.

Another advantage of herbal medicines is their wide availability and cost-effectiveness. Medicinal plants are often easily accessible in rural and remote regions where modern healthcare facilities may be limited. In addition, herbal remedies are frequently more affordable than synthetic pharmaceuticals, making them an important healthcare option for economically disadvantaged populations. Furthermore, herbal medicines have historically demonstrated good patient acceptance due to their long-standing use in traditional medical practices.

However, scientific evaluation of herbal medicines is essential to ensure their safety, efficacy, and quality. Standardization, proper identification of plant materials, and controlled pharmacological studies are necessary to establish the therapeutic potential of herbal products. Integrating traditional herbal knowledge with modern research methodologies can therefore enhance the development of safe and effective plant-based medicines.

3. MATERIALS AND EQUIPMENTS

3.1 Material

Table 3.1: Materials Used and Their Sources.

Sr. No.	Name of Material	Grade	Source / Supplier
1	<i>Gmelina arborea</i> leaves	Dried plant material	Collected locally and authenticated
2	Ethanol	Analytical grade	Merck Life Sciences Pvt. Ltd., India
3	Methanol	Analytical grade	Merck Life Sciences Pvt. Ltd., India
4	Acetone	Analytical grade	Sisco Research Laboratories (SRL), India
5	Chloroform	Analytical grade	HiMedia Laboratories Pvt. Ltd., India
6	Distilled Water	Laboratory grade	Prepared in laboratory (In-house)
7	Gallic Acid	Analytical grade	HiMedia Laboratories Pvt. Ltd., India
8	Quercetin	Analytical grade	HiMedia Laboratories
9	Folin–Ciocalteu reagent	Analytical grade	Merck Life Sciences Pvt. Ltd., India
10	Magnesium ribbon	Analytical grade	SRL Chemicals Pvt. Ltd., India
11	Concentrated Sulphuric Acid	Analytical grade	Merck Life Sciences Pvt. Ltd., India
12	Ferric Chloride	Analytical grade	HiMedia Laboratories Pvt. Ltd., India

3.2 Equipment

Table 3.2: Equipment Used and Their Makers.

Sr. No.	Name of Equipment	Model / Make
1	Soxhlet Extraction Apparatus	Borosil Glass Works Pvt. Ltd., India
2	Rotary Vacuum Evaporator	Heidolph Laborota 4000 Efficient
3	Heating Mantle	Remi Instruments Pvt. Ltd., India
4	Electronic Analytical Balance	Shimadzu Corporation, Japan

5	Hot Air Oven	Remi Instruments Pvt. Ltd., India
6	Mechanical Grinder	Philips Laboratory Grinder
7	Digital pH Meter	Eutech Instruments / Lab India
8	Glassware (Beakers, Flasks, Test tubes etc.)	Borosil Laboratory Glassware

4. Experimental Work

4.1 Acute Oral Toxicity Study (OECD Guideline 423)

The acute oral toxicity study of the extract of *Gmelina arborea* was conducted according to OECD Guideline 423, which is designed to evaluate the toxicological effects of substances following a single-dose oral administration. In this procedure, small groups of animals are treated sequentially with increasing doses of the test substance in order to determine the dose level that may produce toxic effects.

For the present study, three animals were used for each dose level. The extract of *Gmelina arborea* was administered orally at sequential dose levels of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight.

After administration of the extract, the animals were carefully observed for any signs of toxicity or mortality. Particular attention was given to behavioral and physiological parameters such as

- Changes in skin and fur
- Condition of eyes and mucous membranes
- Respiratory pattern
- Salivation
- Tremors and convulsions
- Lethargy or sedation
- Locomotor activity and behavioral abnormalities

The animals were observed continuously during the first four hours after dosing, periodically during the first 24 hours, and thereafter daily for a period of 14 days.

If no mortality or severe toxic symptoms were observed at the lower dose levels, a limit test dose of 2000 mg/kg body weight was administered as recommended by the OECD guideline. The absence of mortality or significant toxic manifestations at this dose indicated that the extract of *Gmelina arborea* is relatively safe and well tolerated within the tested dose range.

4.2 Observation Parameters

During the experimental period, the animals were closely monitored for any signs of toxicity or abnormal physiological responses. Observational parameters included changes in locomotor activity, tremors, convulsions, salivation, diarrhea, lethargy, and any unusual behavioral responses.

In addition to behavioral observations, body weight measurements were recorded on Day 0, Day 7, and Day 14 to evaluate the possible effects of the extract on normal growth and metabolism.

At the end of the observation period, the animals were subjected to gross necropsy examination to identify any abnormalities in major internal organs such as the liver, kidneys, heart, lungs, and spleen. This evaluation helped determine whether the extract produced any adverse physiological effects.

4.3 Selection of Experimental Dose

Based on the results obtained from the acute toxicity study, the median lethal dose (LD₅₀) of the extract of *Gmelina arborea* was found to be greater than 2000 mg/kg body weight. According to standard pharmacological practice, safe experimental doses for further pharmacological studies are usually selected as fractions of the maximum tolerated dose.

Therefore, for the evaluation of anxiolytic activity using Elevated Plus Maze and Light–Dark Box models, two dose levels of the plant extract were selected

- Low dose: 200 mg/kg body weight
- High dose: 400 mg/kg body weight

These doses correspond approximately to 1/10th and 1/5th of the maximum safe dose, ensuring both safety and pharmacological effectiveness during the anxiolytic evaluation.

Experimental Design

Table 4.1: Experimental Design for Acute Toxicity Study.

Group	Number of Animals	Dose (mg/kg)	Route of Administration	Observation Period
Group I	3 animals	5 mg/kg	Oral	14 days
Group II	3 animals	50 mg/kg	Oral	14 days
Group III	3 animals	300 mg/kg	Oral	14 days
Group IV	3 animals	2000 mg/kg	Oral	14 days

4.4 Elevated Plus Maze (EPM) Model

Principle

The Elevated Plus Maze model is one of the most commonly used experimental methods for assessing anxiety-related behavior in rodents. The principle of this model is based on the natural aversion of rodents to open and elevated spaces, combined with their innate tendency to explore unfamiliar environments.

Rodents generally prefer enclosed and protected areas because these provide safety from potential predators. When placed in an elevated maze consisting of both open and closed arms, anxious animals tend to remain in the closed arms and avoid the open arms due to fear of exposure. However, when animals are treated with anxiolytic agents, their anxiety levels decrease and they exhibit increased exploration of the open arms.

Therefore, an increase in the number of entries and time spent in the open arms of the maze is considered indicative of anxiolytic activity.

Apparatus

The Elevated Plus Maze apparatus consists of four arms arranged in the shape of a plus sign (+) and elevated approximately 40–70 cm above the floor. Two of the arms are open without walls, while the other two arms are enclosed by vertical walls. All four arms are connected by a central square platform.

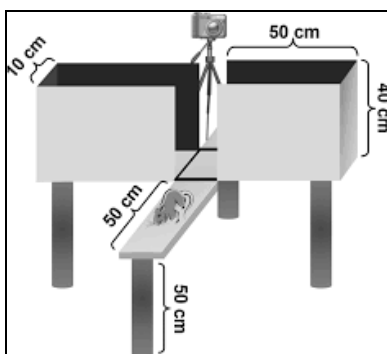


Figure 4.2: Elevated Plus Maze (EPM) Model Apparatus.

The open arms expose the animal to height and light, creating an anxiety-provoking environment, whereas the closed arms provide a sense of safety and protection. The dimensions of the apparatus are typically standardized to ensure consistent experimental conditions.

Experimental Procedure

Each animal was placed individually in the central platform of the maze facing one of the open arms. The animal was then allowed to explore the apparatus freely for a period of five minutes. During this time, the behavior of the animal was carefully observed and recorded.

After each trial, the apparatus was cleaned with alcohol solution to eliminate any odor cues that could influence the behavior of subsequent animals.

Behavioral Parameters Recorded

The following behavioral parameters were recorded during the experiment:

- Number of entries into the open arms
- Number of entries into the closed arms
- Time spent in the open arms
- Time spent in the closed arms

An increase in the number of entries and time spent in the open arms indicated a reduction in anxiety levels and suggested anxiolytic activity of the test extract.

4.5 Light–Dark Box (LDB) Model

Principle

The Light–Dark Box test is another widely used behavioral model for evaluating anxiety in rodents. The principle of this model is based on the natural preference of rodents for dark, enclosed spaces and their avoidance of brightly illuminated environments.

Rodents have an inherent tendency to avoid bright light because it increases their vulnerability to predators. At the same time, they possess a natural exploratory drive that encourages them to investigate new environments. This creates a behavioral conflict between the tendency to explore and the fear of open or illuminated spaces.

Anxiolytic agents reduce the fear response and increase the exploratory behavior of animals in illuminated areas. Therefore, animals treated with anxiolytic substances tend to spend more time in the light compartment and exhibit increased movement between the compartments.

Apparatus

The Light–Dark Box apparatus consists of two compartments of equal size: one brightly illuminated chamber and one dark enclosed chamber. These compartments are connected by a small opening or doorway that allows the animal to move freely between the two sections.

The light compartment is usually made of transparent or white material and is brightly illuminated, while the dark compartment is covered and provides minimal light.



Figure 4.3: Light–Dark Box (LDB) Model Apparatus.

Experimental Procedure

During the experiment, each animal was placed in the center of the light compartment facing away from the dark chamber. The animal was then allowed to explore both compartments freely for a period of five minutes.

The movements and behavioral responses of the animals were carefully observed and recorded during the test period.

After each experiment, the apparatus was cleaned thoroughly to remove any odor traces from previous animals.

Behavioral Parameters Recorded

The following parameters were recorded during the Light–Dark Box test

- Time spent in the light compartment
- Time spent in the dark compartment
- Number of transitions between the compartments
- Latency to enter the dark chamber

Animals with lower anxiety levels tend to spend more time in the light compartment and exhibit increased exploratory activity between the two compartments. Therefore, increased time spent in the illuminated area and higher transition frequency indicate anxiolytic activity.

4.6 Experimental Design for Evaluation of Anxiolytic Activity

The anxiolytic activity of the extract of *Gmelina arborea* was evaluated using two well-established behavioral models, namely the Elevated Plus Maze (EPM) and the Light–Dark

Box (LDB) test. These models are widely used in neuropharmacological research to assess anxiety-related behaviors in rodents.

For the experiment, healthy adult experimental animals were randomly divided into different groups to evaluate the anxiolytic potential of the plant extract. Randomization was performed to minimize experimental bias and ensure uniform distribution of animals across all groups.

The animals were divided into four experimental groups, with six animals in each group.

Experimental Groups

Group I – Control Group: Animals in this group received the vehicle (distilled water or suitable solvent) orally and served as the normal control.

Group II – Standard Drug Group: Animals in this group received a standard anxiolytic drug such as diazepam (1 mg/kg body weight, p.o.), which served as the positive control for comparison with the test extract.

Group III – Low Dose Test Group: Animals in this group received the extract of *Gmelina arborea* at a dose of 200 mg/kg body weight administered orally.

Group IV – High Dose Test Group: Animals in this group received the extract of *Gmelina arborea* at a dose of 400 mg/kg body weight administered orally.

The test extract and standard drug were administered orally using a gastric gavage needle approximately 30–60 minutes before the behavioral tests.

Following treatment, the animals were individually subjected to behavioral evaluation using the Elevated Plus Maze (EPM) and Light–Dark Box (LDB) models. The experiments were conducted in a quiet laboratory environment to minimize external disturbances that could influence the behavior of the animals.

The behavioral parameters recorded during these tests were used to assess the anxiolytic activity of the plant extract by comparing the results obtained from the treated groups with those of the control and standard drug groups.

Table 4.2: Experimental Design for Anxiolytic Activity Study.

Group	Treatment	Dose	Number of Animals	Route
Group I	Control (Vehicle)	—	6	Oral
Group II	Standard Drug (Diazepam)	1 mg/kg	6	Oral
Group III	<i>Gmelina arborea</i> Extract (Low Dose)	200 mg/kg	6	Oral
Group IV	<i>Gmelina arborea</i> Extract (High Dose)	400 mg/kg	6	Oral

4.7 Statistical Analysis

The data obtained from the behavioral experiments were expressed as mean $\pm$ standard error of mean (SEM) for each experimental group. Statistical analysis was performed to determine the significance of differences between the control group, standard drug group, and test groups treated with different doses of the plant extract. The statistical evaluation of the experimental data was carried out using one-way Analysis of Variance (ANOVA). This method is commonly used to compare the means of multiple groups and determine whether any significant differences exist among them.

After performing ANOVA, Dunnett's multiple comparison test was applied as a post-hoc test to compare each treated group with the control group. Dunnett's test is particularly useful in pharmacological studies where multiple treatment groups are compared with a single control group.

5. RESULT AND DISCUSSION

5.1 Extraction of Plant Material

The dried and powdered plant material of *Gmelina arborea* was subjected to extraction using the Soxhlet extraction technique. Soxhlet extraction is a widely used method in phytochemical research because it allows continuous extraction of plant constituents using a suitable organic solvent.

In the present study, accurately weighed powdered plant material was placed in a Soxhlet apparatus and extracted using ethanol as the extraction solvent. Ethanol was selected because it possesses good solubility for a wide range of phytoconstituents including flavonoids, phenolic compounds, alkaloids, and glycosides.

The extraction process was continued for several cycles until the solvent in the siphon tube became colorless, indicating complete extraction of soluble constituents from the plant material. The obtained extract was filtered using Whatman filter paper to remove insoluble residues.

The filtrate was concentrated under reduced pressure using a rotary evaporator to remove the solvent and obtain a semi-solid extract. The concentrated extract was further dried in a desiccator to remove residual moisture. The final dried extract was weighed and the percentage yield was calculated.

Table 5.1: Extraction Yield of *Gmelina arborea*.

Parameter	Value
Weight of plant powder used for extraction	500 g
Weight of extract obtained	62.5 g
Extraction solvent	Ethanol
Extraction method	Soxhlet extraction
Percentage yield	12.5 %

5.2 Percentage Yield

The extraction process yielded **12.5% w/w of extract**, indicating efficient recovery of phytoconstituents from the plant material.

The processing and extraction of *Gmelina arborea* leaves resulted in a stable crude extract suitable for further phytochemical and pharmacological investigations. The obtained percentage yield indicates that the Soxhlet extraction method using ethanol was effective in isolating bioactive constituents from the plant material. The extract obtained was stored under controlled conditions and used for subsequent phytochemical screening and evaluation of anxiolytic activity.

5.3 Physicochemical Evaluation of *Gmelina arborea* Leaf Powder

Physicochemical evaluation of the powdered leaves of *Gmelina arborea* was performed to determine the quality, purity, and standardization parameters of the crude drug. Parameters such as extractive values, ash values, and moisture content were evaluated according to standard pharmacopoeial procedures. These tests provide important information regarding the presence of soluble phytoconstituents, inorganic impurities, and the stability of the crude drug. The obtained results indicated that the plant material contained a considerable amount of alcohol-soluble phytoconstituents and minimal inorganic impurities. The moisture content was also within acceptable limits, suggesting proper drying and storage of the crude drug.

5.4 Extractive Values

Extractive values indicate the amount of active phytoconstituents that can be extracted using specific solvents. In the present study, alcohol and water were used as extraction solvents to determine the extractive values of *Gmelina arborea* leaf powder. The alcohol-soluble extractive value was found to be higher than the water-soluble extractive value, suggesting the presence of moderately polar phytoconstituents such as flavonoids and phenolic compounds.

5.6 Ash Values

Ash values represent the amount of inorganic residue remaining after complete incineration of the plant material. These values help identify the presence of mineral content and extraneous inorganic matter. The results showed that the total ash value of the plant material was moderate, while the acid-insoluble ash value was relatively low, indicating minimal contamination with silica or earthy matter.

Loss on Drying

Loss on drying was determined to estimate the moisture content present in the crude plant material. The moisture content was found to be within acceptable pharmacognostic limits, indicating that the plant material was adequately dried and suitable for storage and further analysis.

Table 5.1: Physicochemical Parameters of *Gmelina arborea* Leaf Powder.

Parameter	Observation	Value (% w/w)
Alcohol soluble extractive value	Moderate	15.8 %
Water soluble extractive value	Moderate	12.4 %
Total ash value	Inorganic residue	8.6 %
Acid insoluble ash	Silica and earthy matter	1.9 %
Water soluble ash	Water soluble salts	3.4 %
Loss on drying	Moisture content	7.6 %

The physicochemical parameters obtained for *Gmelina arborea* leaf powder fall within acceptable limits reported for medicinal plants. The higher alcohol-soluble extractive value indicates the presence of bioactive phytoconstituents soluble in organic solvents. The low acid-insoluble ash value suggests minimal contamination with inorganic impurities, while the moderate moisture content indicates proper drying and storage conditions. These results confirm the quality and authenticity of the plant material used for further phytochemical and pharmacological investigations.

Phytochemical Screening of *Gmelina arborea* Extract

Preliminary phytochemical screening of the ethanolic extract of *Gmelina arborea* leaves was carried out to identify the presence of various bioactive secondary metabolites. These phytoconstituents are responsible for many pharmacological activities of medicinal plants, including antioxidant, neuroprotective, and anxiolytic effects. The qualitative phytochemical analysis was performed using standard chemical tests for different classes of compounds such as alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, carbohydrates, proteins, and phenolic compounds.

The results of phytochemical screening revealed the presence of several important secondary metabolites in the extract. Compounds such as flavonoids, tannins, phenolic compounds, glycosides, and saponins were found to be present in the plant extract, which may contribute to its pharmacological activity. However, proteins and amino acids were found to be absent or present in trace amounts. The presence of these phytoconstituents suggests that *Gmelina arborea* may possess significant therapeutic potential.

Table 5.2: Phytochemical Screening of *Gmelina arborea* Extract.

Phytochemical Constituents	Test Performed	Result
Alkaloids	Mayer's, Wagner's test	Present (+)
Carbohydrates	Molisch's test	Present (+)
Proteins	Biuret test	Absent (–)
Amino acids	Ninhydrin test	Absent (–)
Flavonoids	Shinoda test	Present (+)
Tannins	Ferric chloride test	Present (+)
Phenolic compounds	Ferric chloride test	Present (+)
Glycosides	Keller–Killiani test	Present (+)
Saponins	Foam test	Present (+)
Steroids	Liebermann–Burchard test	Present (+)
Terpenoids	Salkowski test	Present (+)

The preliminary phytochemical screening confirmed that the ethanolic extract of *Gmelina arborea* leaves contains several biologically active constituents, including flavonoids, phenolics, glycosides, and saponins. These compounds are widely reported for their antioxidant, anti-inflammatory, and neuroprotective activities. The presence of these phytoconstituents may contribute to the anxiolytic potential of the plant extract observed in subsequent pharmacological studies.

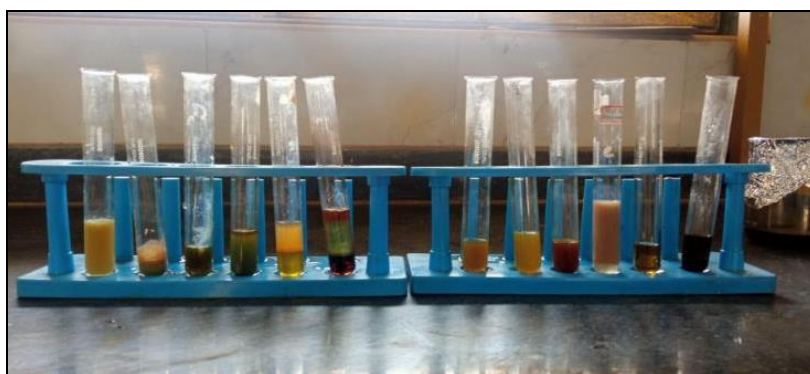


Figure 5.1: preliminary phytochemical evaluation.

Quantitative Evaluation of Ethanolic Extract of *Gmelina arborea* Leaves

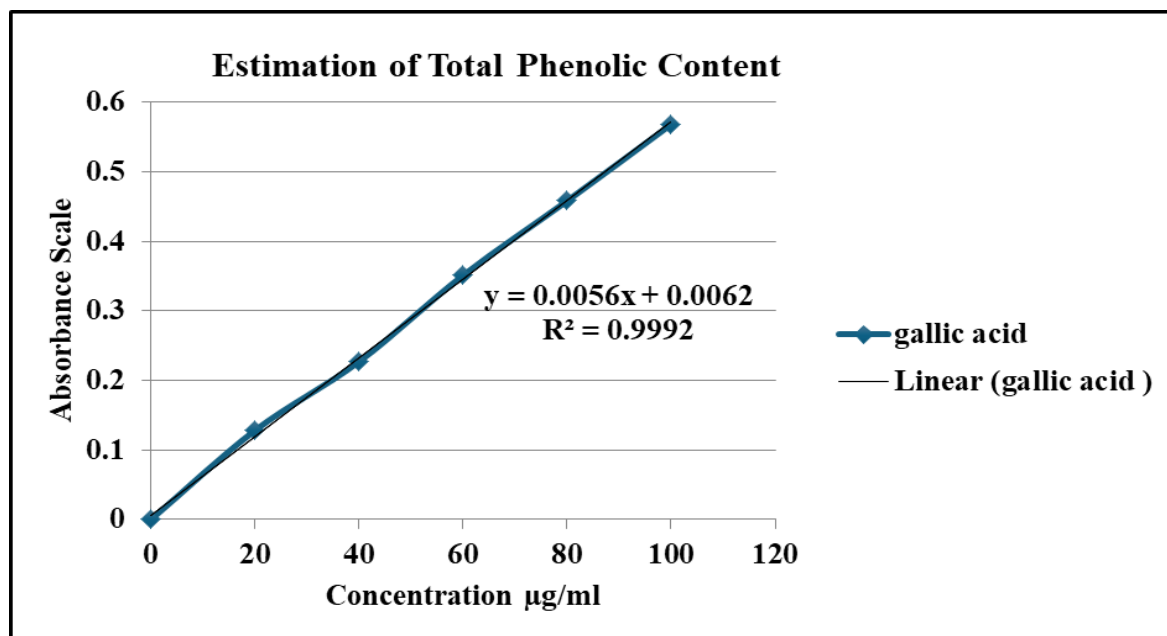
Quantitative Analysis of Total Phenolic and Flavonoid Contents

The quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was performed to determine the concentration of important phytochemicals present in the ethanolic extract of *Gmelina arborea* leaves (GGLE). Phenolic compounds and flavonoids are known for their strong antioxidant and neuroprotective properties and may contribute significantly to the anxiolytic activity of medicinal plants.

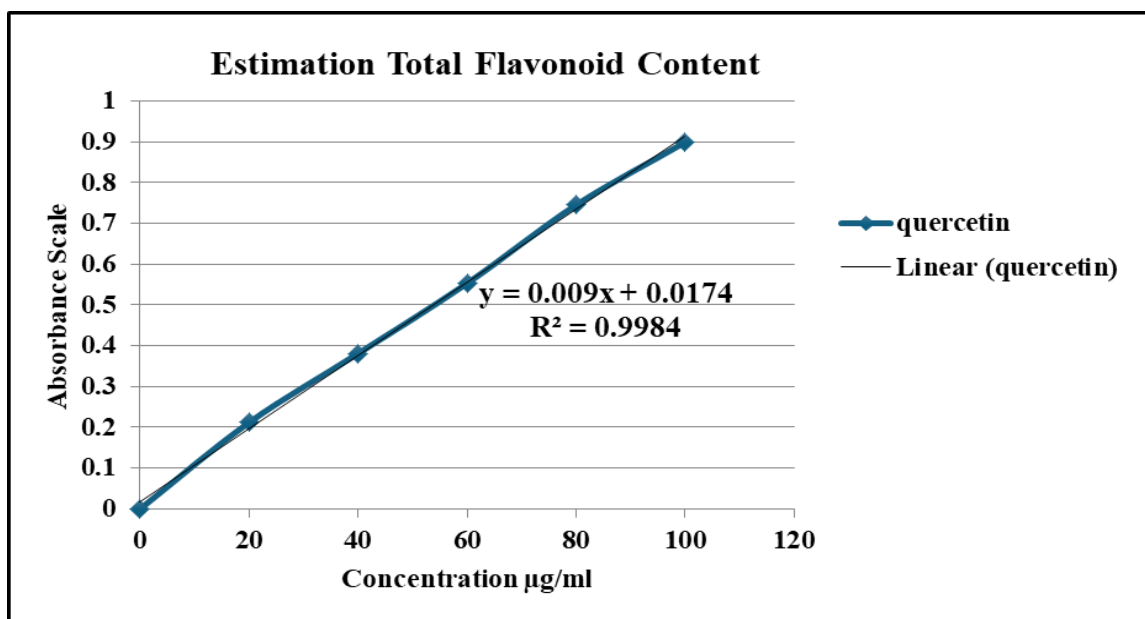
The results of quantitative analysis revealed that the ethanolic extract of *Gmelina arborea* contained 27.832 ± 0.42 mg gallic acid equivalents per gram (mg GAE/g) of phenolic compounds and 48.67 ± 0.35 mg quercetin equivalents per gram (mg QE/g) of flavonoids. These values indicate the presence of a considerable amount of phenolic and flavonoid constituents in the extract, suggesting potential pharmacological benefits.

Table 5.2: Quantitative analysis of ethanolic extract of *Gmelina arborea*.

Sr. No.	Extract	Total Phenol (mg GAE/g)	Total flavonoid (quercetin equivalent/g)
1.	Ethanolic extract of <i>Gmelina arborea</i>	27.832 ± 0.42	48.67 ± 0.35



(a)



(b)

Figure 5.3: (a) Estimation of Total Phenolic Content Graph (b) Estimation of Total Flavonoids Content Graph.

Alkaloid Quantification

The alkaloid content of *Gmelina arborea* leaf extract was determined using the Harborne method. This method involves extraction of alkaloids through sequential acidic and basic solvent treatments followed by spectrophotometric analysis. A standard calibration curve was prepared using a reference alkaloid compound with concentrations ranging from 10–50 µg/mL, which demonstrated a linear relationship between absorbance and concentration at 470 nm.

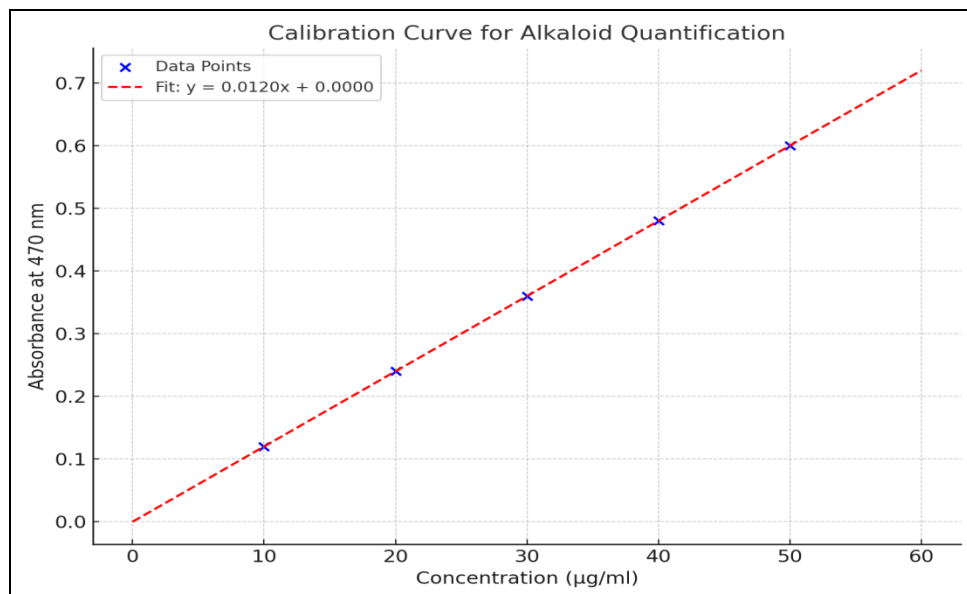
The ethanolic extract of *Gmelina arborea* leaves showed an absorbance value of 0.528 at 470 nm, corresponding to an alkaloid content of 35.41 mg/g extract as determined from the calibration curve. These results indicate that the plant extract contains a significant amount of alkaloids, which are known for various pharmacological activities including central nervous system modulation.

Table 5.4: Observation Table for Alkaloid Content Analysis.

Concentration of Standard (µg/mL)	Absorbance (470 nm)
10	0.152
20	0.305
30	0.459
40	0.611
50	0.764

Table 5.5: Results of Alkaloid Content in Ethanolic extract of *Gmelina arborea*.

Sample	Absorbance (470 nm)	Alkaloid Content (mg/g extract)
Ethanolic extract of <i>Gmelina arborea</i>	0.528	35.41

**Figure 5.4: Calibration curve for Alkaloid Quantification.**

Tannin Content Assessment of Ethanolic extract of *Gmelina arborea*

The tannin content present in the ethanolic extract of *Gmelina arborea* leaves was quantified using the Vanillin–HCl method. In this method, a calibration curve was prepared using catechin as the standard compound, and the tannin content was expressed as milligrams of catechin equivalents (CE) per gram of extract.

The ethanolic extract exhibited an absorbance value corresponding to a tannin content of 47.89 mg CE/g extract at 500 nm. The presence of a significant amount of tannins indicates the potential pharmacological importance of the plant extract, as tannins possess antioxidant and protective biological properties.

Table 5.6: Observation Table for Catechin Standard.

Concentration (µg/mL)	Absorbance (500 nm)
10	0.145
20	0.290
40	0.575
60	0.860
80	1.155
100	1.430

Table 5.7: Results of Tannin Content in Ethanolic extract of *Gmelina arborea*.

Sample	Absorbance (500 nm)	Tannin Content (mg CE/g extract)
Ethanolic extract of <i>Gmelina arborea</i>	0.710	47.89

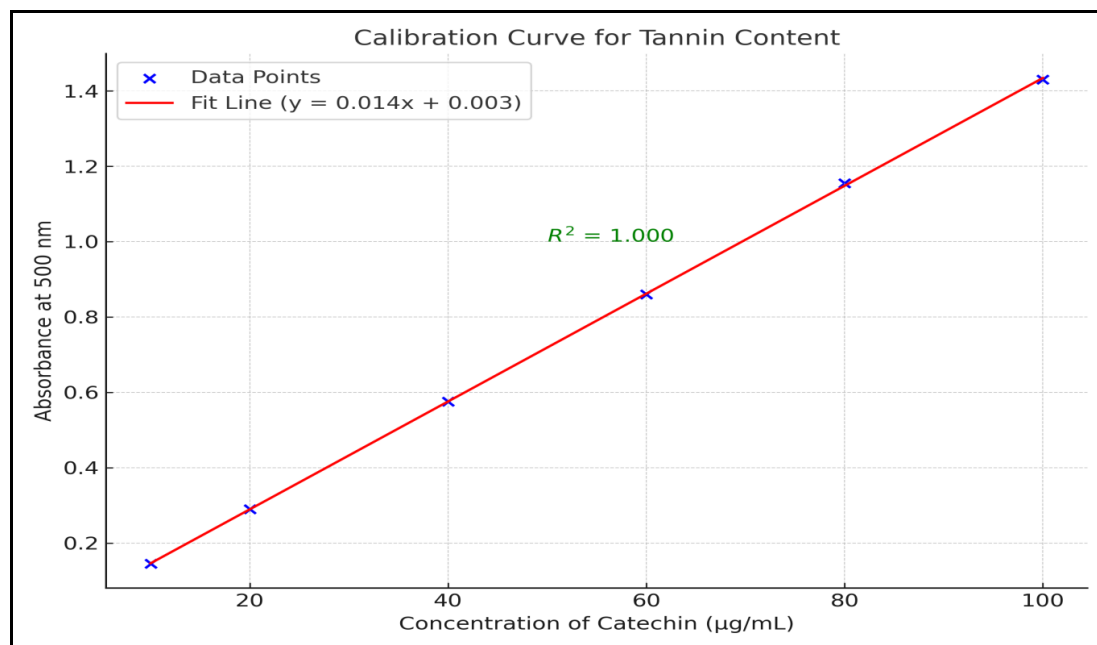


Figure 8.5: Calibration Curve for Tannin Content.

Terpenoid Content in *Gmelina arborea* Extract

The terpenoid content of the methanolic extract of *Gmelina arborea* was quantified using the Salkowski method. The formation of a reddish-brown layer at the chloroform–sulfuric acid interface confirmed the presence of terpenoids in the extract. The absorbance of the sample was measured at 490 nm.

A calibration curve was prepared in the concentration range of 20–100 µg/mL, yielding the regression equation:

$$y = 0.0064x + 0.001$$

with a correlation coefficient ($R^2 > 0.99$), indicating good linearity and reliability of the analytical method. The absorbance value of the plant extract was 0.320, which corresponded to a terpenoid concentration of 49.84 µg/mL.

These results demonstrate that the extract contains a substantial amount of terpenoids, which are known for their diverse pharmacological properties including anti-inflammatory, antioxidant, and neuroprotective activities.

Table 5.8: Observation table for calibration curve.

Concentration ($\mu\text{g/mL}$)	Absorbance at 490 nm
20	0.128
40	0.256
60	0.384
80	0.512
100	0.640

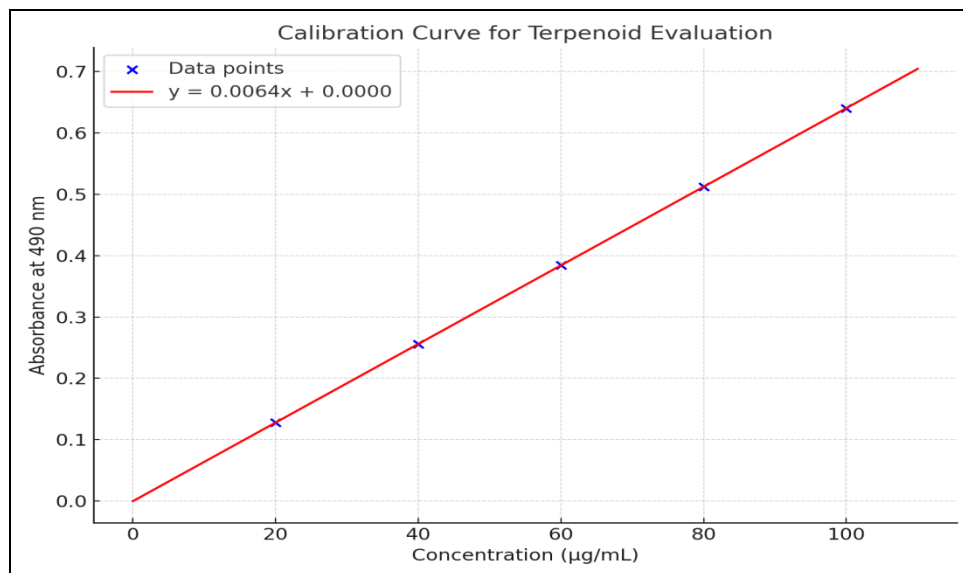


Figure 5.9: Calibration curve of Terpenoid Evaluation.

Pharmacological Study

Acute Toxicity Study

The acute oral toxicity study of the ethanolic extract of *Gmelina arborea* leaves was conducted in accordance with **OECD Guideline 423 (Acute Toxic Class Method)** to determine the safety profile and maximum tolerated dose of the extract prior to its pharmacological evaluation. The test extract was administered orally to experimental animals at sequential dose levels of **5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight**, and the animals were observed for signs of toxicity and mortality.

During the experimental period, all animals were carefully monitored for behavioral changes and physiological abnormalities. The observations included changes in skin and fur, eye and mucous membrane appearance, respiratory patterns, salivation, tremors, convulsions, lethargy, and locomotor activity.

No mortality or significant toxic symptoms were observed in animals treated with the extract at any of the tested dose levels throughout the **14-day observation period**. The animals

maintained normal behavioral patterns and physiological responses during the study. These findings indicated that the extract of *Gmelina arborea* was safe and well tolerated at the tested doses.

Table 5.10: Experimental Design for Acute Toxicity Study.

Group	Number of Animals	Dose (mg/kg)	Route of Administration	Observation Period
Group I	3 animals	5 mg/kg	Oral	14 days
Group II	3 animals	50 mg/kg	Oral	14 days
Group III	3 animals	300 mg/kg	Oral	14 days
Group IV	3 animals	2000 mg/kg	Oral	14 days

Table 5.11: Observations of Toxicity Signs in Experimental Animals.

Dose (mg/kg)	Mortality	Skin/Fur Changes	Tremors/Convulsions	Salivation	Locomotor Activity	Behavioral Changes
5 mg/kg	None	Normal	None	None	Normal	None
50 mg/kg	None	Normal	None	None	Normal	None
300 mg/kg	None	Normal	None	None	Normal	None
2000 mg/kg	None	Normal	None	None	Normal	None

The results indicated that the ethanolic extract of *Gmelina arborea* did not produce any toxic manifestations or mortality in experimental animals even at the highest tested dose of **2000 mg/kg body weight**.

Effect on Body Weight of Animals

Body weight measurements were recorded during the experimental period to evaluate the effect of the extract on the normal growth and metabolic activity of the animals. The results indicated that animals in all treatment groups exhibited a gradual increase in body weight during the study period, suggesting that the extract did not adversely affect normal physiological functions.

Table 8.12: Body Weight Changes During Acute Toxicity Study.

Dose (mg/kg)	Body Weight (Day 0) (g)	Body Weight (Day 7) (g)	Body Weight (Day 14) (g)
5 mg/kg	168 ± 3.2	171 ± 3.4	175 ± 3.6
50 mg/kg	170 ± 2.9	174 ± 3.1	178 ± 3.5
300 mg/kg	169 ± 3.1	173 ± 3.3	177 ± 3.4
2000 mg/kg	171 ± 3.0	175 ± 3.2	179 ± 3.7

The progressive increase in body weight across all experimental groups confirmed that the plant extract did not produce any adverse effects on the general health of the animals.

The acute toxicity study revealed that the ethanolic extract of *Gmelina arborea* was **safe up to a dose of 2000 mg/kg body weight**, as no mortality or toxic symptoms were observed in the experimental animals. According to OECD guidelines, this indicates that the **LD₅₀ value of the extract is greater than 2000 mg/kg body weight**.

Based on these findings, two safe doses of the plant extract were selected for further pharmacological evaluation of anxiolytic activity:

- **Low dose:** 200 mg/kg body weight
- **High dose:** 400 mg/kg body weight

These doses represent approximately **1/10th and 1/5th of the maximum tolerated dose**, ensuring safety during behavioral studies using the **Elevated Plus Maze and Light–Dark Box models**.

***In-Vivo* Pharmacological Evaluation of Anxiolytic Activity**

Elevated Plus Maze Test

The anxiolytic activity of the ethanolic extract of *Gmelina arborea* leaves was evaluated using the **Elevated Plus Maze (EPM) model**, which is one of the most widely used behavioral models for assessing anxiety-related behavior in rodents. The principle of this test is based on the natural aversion of rodents to open and elevated spaces and their preference for enclosed arms. Anxiolytic agents are known to increase exploratory behavior in the open arms of the maze, resulting in increased time spent and number of entries in the open arms.

In the present study, the effects of *Gmelina arborea* extract at two dose levels were compared with the control group and the standard drug **diazepam (1 mg/kg)**.

Table 8.13: Effect of *Gmelina arborea* Extract on Elevated Plus Maze Parameters.

Treatment	Time Spent in Open Arm (sec)	Time Spent in Enclosed Arm (sec)	No. of Entries in Open Arm	No. of Entries in Enclosed Arm	Time Spent in Central Zone (sec)
Group I Vehicle (DW)	104.8 ± 3.12	164.1 ± 5.1	6.6 ± 1.6	9 ± 3.1	17.1 ± 1.9
Group II Standard Drug Diazepam (1 mg/kg)	204 ± 4.5	54.6 ± 7.5	25.5 ± 4.6	10 ± 0.89	26 ± 3.6
Group III <i>Gmelina arborea</i> Extract (Low Dose 100 mg/kg)	141.5 ± 3.3	142.1 ± 6.0	16 ± 3.6	7.8 ± 1.16	14.83 ± 1.6
Group IV <i>Gmelina arborea</i> Extract (High Dose 200 mg/kg)	166 ± 8.7	113 ± 7.5	21.3 ± 1.8	8.5 ± 1.04	21.16 ± 2.7

* $p < 0.001$, $p < 0.05$ vs Vehicle (One-way ANOVA followed by Tukey's test)

The results obtained from the Elevated Plus Maze test demonstrated a significant anxiolytic effect of the ethanolic extract of *Gmelina arborea*. Animals in the **control group** spent more time in the enclosed arms (164.1 ± 5.1 sec) compared to the open arms (104.8 ± 3.12 sec), indicating a natural avoidance of open spaces due to anxiety-related behavior.

Administration of the **standard drug diazepam (1 mg/kg)** produced a marked anxiolytic effect, as evidenced by a substantial increase in the time spent in the open arms (204 ± 4.5 sec) and a reduction in time spent in the enclosed arms (54.6 ± 7.5 sec). Additionally, the number of entries into the open arms increased significantly (25.5 ± 4.6), indicating enhanced exploratory activity.

Treatment with *Gmelina arborea* extract at **100 mg/kg** resulted in a noticeable increase in the time spent in the open arms (141.5 ± 3.3 sec) compared to the control group. This group also showed an increased number of entries into the open arms (16 ± 3.6), suggesting a moderate anxiolytic effect of the plant extract.

Similarly, animals treated with the **higher dose of *Gmelina arborea* extract (200 mg/kg)** exhibited a further increase in the time spent in the open arms (166 ± 8.7 sec) and a decrease in time spent in the enclosed arms (113 ± 7.5 sec). The number of entries into the open arms also increased (21.3 ± 1.8), indicating enhanced exploratory behavior and reduced anxiety.

Furthermore, the time spent in the **central zone** was also increased in the treated groups compared to the control group, suggesting increased locomotor activity and exploratory behavior.

Overall, the results indicated that the ethanolic extract of *Gmelina arborea* produced a **dose-dependent anxiolytic effect**, as demonstrated by increased time spent and entries in the open arms of the maze. Although the effect was slightly lower than that observed with the standard drug diazepam, the extract showed significant anxiolytic potential in the Elevated Plus Maze model.

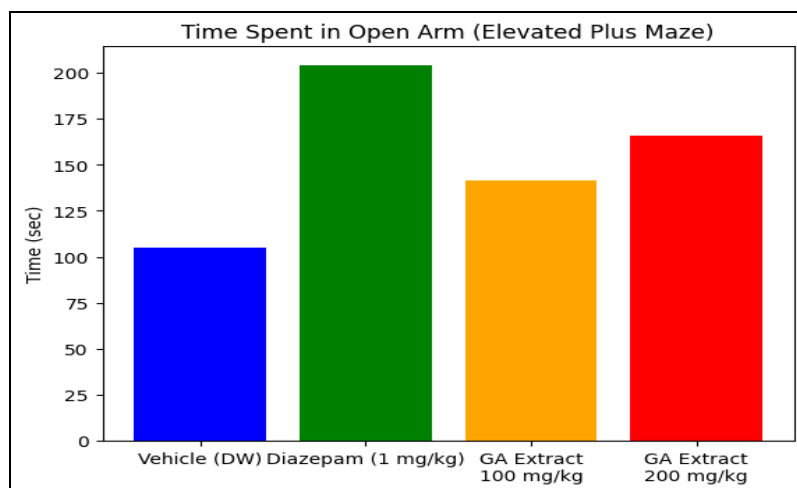


Figure 5.7: Time spent in open arm (in sec) in elevated plus maze test.

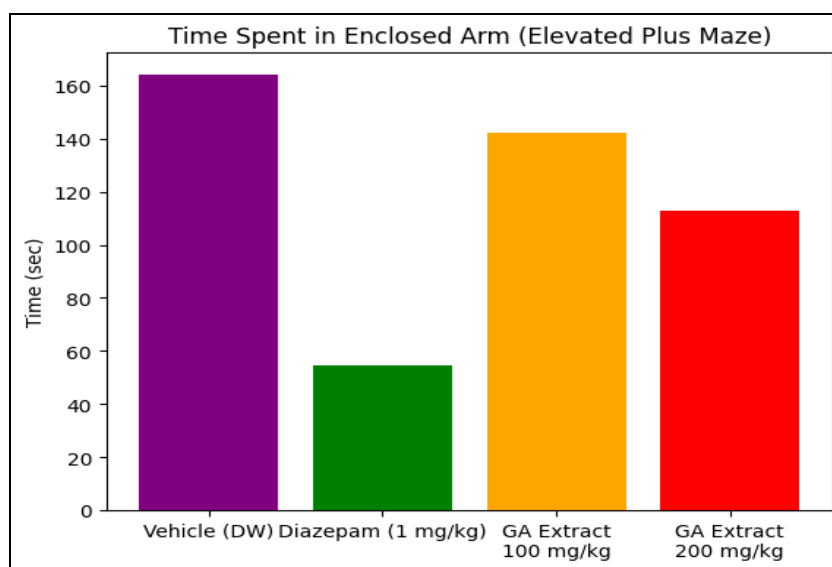


Figure 5.8: Time spent in enclosed arm (in sec) in elevated plus maze test.

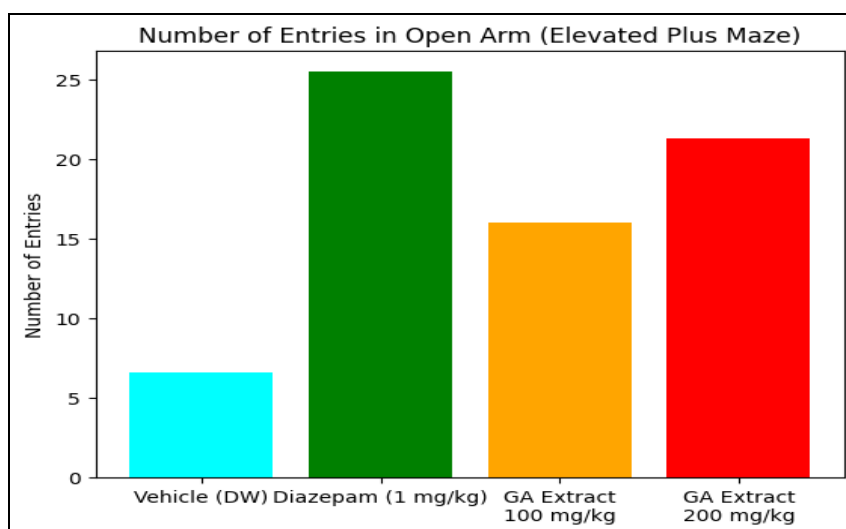


Figure 5.9: Total No. of entries in open arm in elevated plus maze test.

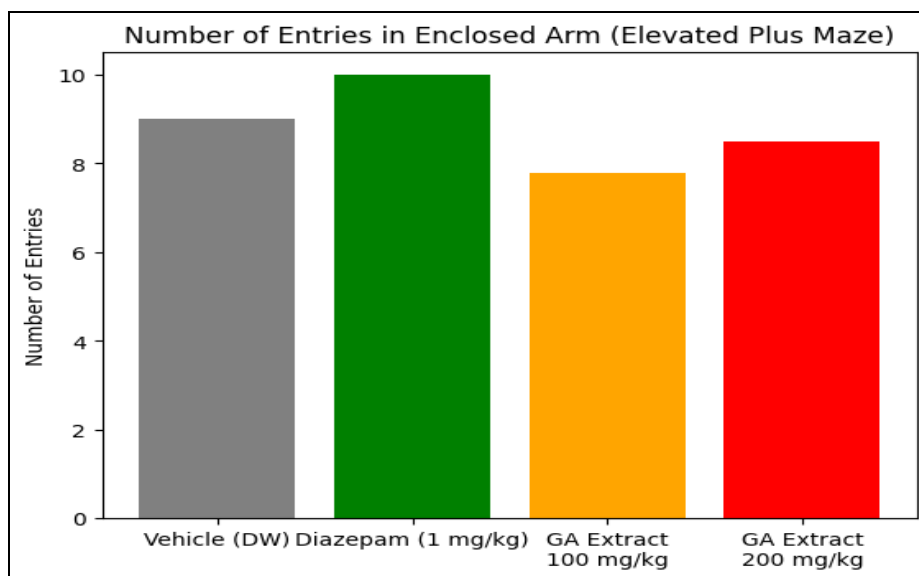


Figure 5.10: Total No. Of entries in enclosed arm in elevated plus maze test.

Elevated Plus Maze Test

The anxiolytic activity of *Gmelina arborea* extract was evaluated using the Elevated Plus Maze (EPM) model. The EPM test is based on the natural tendency of rodents to avoid open and elevated spaces while preferring enclosed areas. Anxiolytic agents increase exploratory behavior in open arms, resulting in increased time spent and entries in open arms.

The **vehicle-treated control group** showed typical anxiety behavior, spending more time in the enclosed arms (164.1 sec) compared to the open arms (104.8 sec). The number of entries into the open arms was also relatively low (6.6), indicating a higher anxiety level in untreated animals.

Animals treated with the **standard drug diazepam (1 mg/kg)** showed a marked anxiolytic effect. The time spent in the open arms increased significantly to **204 sec**, while the time spent in the enclosed arms decreased to **54.6 sec**. The number of entries into the open arms increased to **25.5**, demonstrating enhanced exploratory activity and reduced anxiety.

Treatment with *Gmelina arborea* **extract at a dose of 100 mg/kg** resulted in a moderate increase in the time spent in the open arms (141.5 sec) and a reduction in time spent in the enclosed arms (142.1 sec). The number of entries into the open arms also increased (16), indicating a partial anxiolytic effect of the extract.

Similarly, animals treated with the **higher dose of *Gmelina arborea* extract (200 mg/kg)** exhibited a greater anxiolytic response. The time spent in the open arms increased to **166 sec**,

while the time spent in the enclosed arms decreased to **113 sec**. The number of entries into the open arms increased to **21.3**, indicating improved exploratory behavior and reduced anxiety.

The **time spent in the central zone** also showed variation among groups, with the standard drug group showing the highest value (26 sec). The high-dose extract group also showed increased time in the central zone compared to the control group, suggesting enhanced locomotor and exploratory activity.

Overall, the results demonstrated that the ethanolic extract of *Gmelina arborea* produced a **dose-dependent anxiolytic effect** in the Elevated Plus Maze model, although the effect was slightly lower than that observed with the standard drug diazepam.

Table 5.10: Effect of *Gmelina arborea* Extract on Elevated Plus Maze Parameters.

Treatment	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	No. of entries in open arm	No. of entries in enclosed arm	Time spent in central zone (sec)
Group I Vehicle (DW)	104.8	164.1	6.6	9	17.1
Group II Standard Drug Diazepam (1 mg/kg)	204	54.6	25.5	10	26
Group III <i>Gmelina arborea</i> Extract (Low Dose 100 mg/kg)	141.5	142.1	16	7.8	14.83
Group IV <i>Gmelina arborea</i> Extract (High Dose 200 mg/kg)	166	113	21.3	8.5	21.16

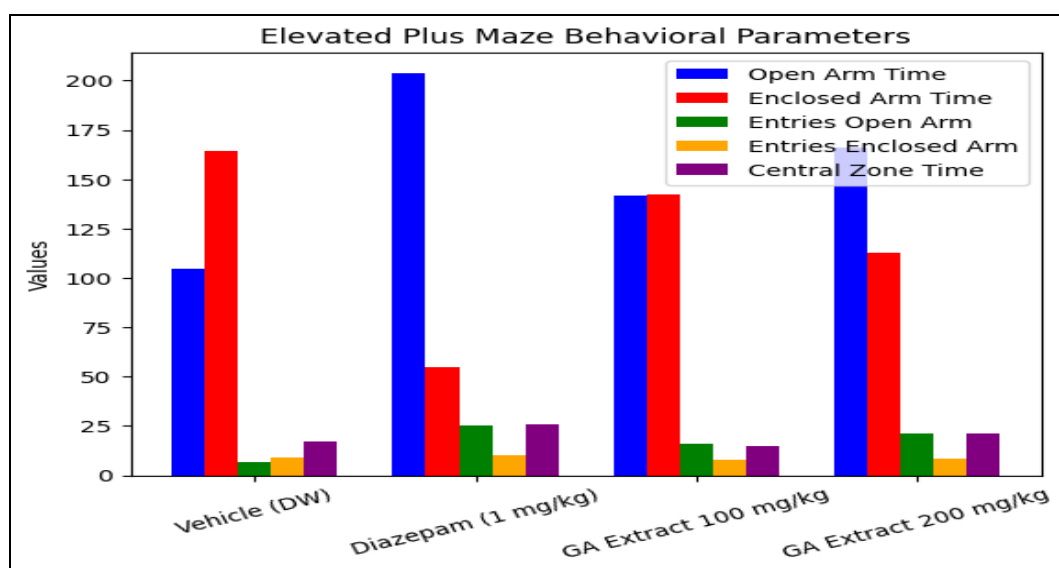


Figure 5.10: Elevated plus maze behavioral parameters.

Light–Dark Box Test

The anxiolytic activity of the ethanolic extract of *Gmelina arborea* was further evaluated using the **Light–Dark Box (LDB) model**, which is a widely used behavioral test for assessing anxiety in rodents. This model is based on the natural tendency of rodents to avoid brightly illuminated areas and prefer dark compartments. Anxiolytic agents typically increase exploratory behavior in the light compartment and increase the number of transitions between the two compartments.

Table 5.11: Effect of *Gmelina arborea* Extract on Light–Dark Box Parameters.

Treatment	No. of Crossings	Time spent in dark zone (sec)	Time spent in light zone (sec)	Transfer latency (sec)
Group I Vehicle (DW)	13 ± 2.2	191.6 ± 4.17	104.8 ± 4.2	22.16 ± 2
Group II Standard Drug Diazepam (1 mg/kg)	32.33 ± 2.8	87.5 ± 5.6	203.1 ± 3.6	26.5 ± 1.5
Group III <i>Gmelina arborea</i> Extract (Low Dose 100 mg/kg)	20 ± 1.4	160.3 ± 5.5	132.5 ± 4	22.5 ± 1.8
Group IV <i>Gmelina arborea</i> Extract (High Dose 200 mg/kg)	22.5 ± 2.1	159.5 ± 4.8	143.16 ± 4	24.16 ± 2.3

Values are expressed as Mean ± SEM (n = 6), * $p < 0.001$, $p < 0.05$ vs Vehicle (One-way ANOVA followed by Tukey's test)

The control group treated with the vehicle (distilled water) exhibited typical anxiety behavior, characterized by fewer crossings between compartments (13 ± 2.2) and more time spent in the dark zone (191.6 ± 4.17 sec) compared to the light zone. This behavior reflects the natural preference of rodents for darker and safer environments when anxiety levels are high.

Animals treated with the standard anxiolytic drug diazepam (1 mg/kg) showed a marked anxiolytic response. The number of crossings increased significantly to 32.33 ± 2.8, indicating enhanced exploratory behavior. In addition, the animals spent more time in the light compartment (203.1 ± 3.6 sec) and less time in the dark compartment (87.5 ± 5.6 sec), confirming the anxiolytic effect of the standard drug.

Treatment with *Gmelina arborea* extract at the low dose (100 mg/kg) resulted in a moderate increase in the number of crossings (20 ± 1.4) compared with the control group. The animals also showed an increase in the time spent in the light compartment (132.5 ± 4 sec) and a corresponding reduction in time spent in the dark zone, suggesting a reduction in anxiety levels.

Similarly, animals treated with the higher dose of *Gmelina arborea* extract (200 mg/kg) exhibited a further increase in exploratory activity with 22.5 ± 2.1 crossings. The time spent in the light compartment increased to 143.16 ± 4 sec, while the time spent in the dark zone decreased compared to the control group. These findings indicate a dose-dependent anxiolytic effect of the plant extract.

The transfer latency values also increased slightly in the treated groups compared to the control group, indicating improved exploratory behavior and reduced anxiety responses.

Overall, the results suggest that the ethanolic extract of *Gmelina arborea* possesses significant anxiolytic activity, as evidenced by increased crossings and increased time spent in the illuminated compartment in the Light–Dark Box model.

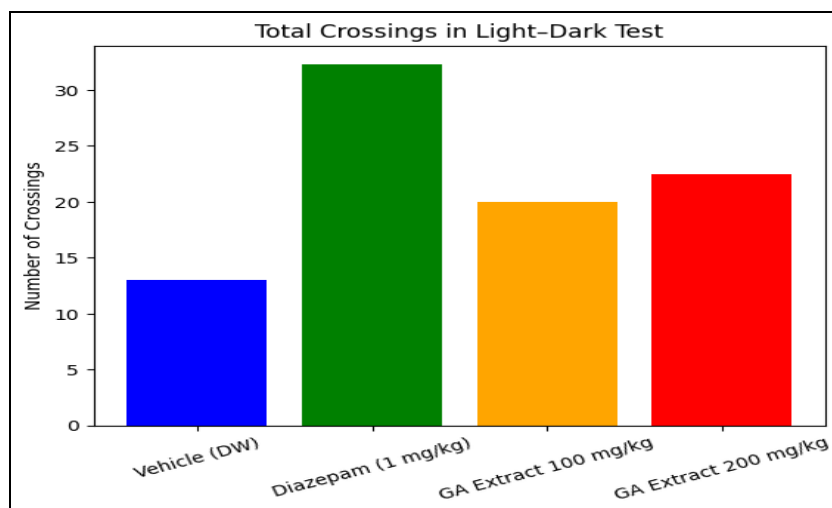


Figure 5.12: Total Crossings in Light–Dark Test.

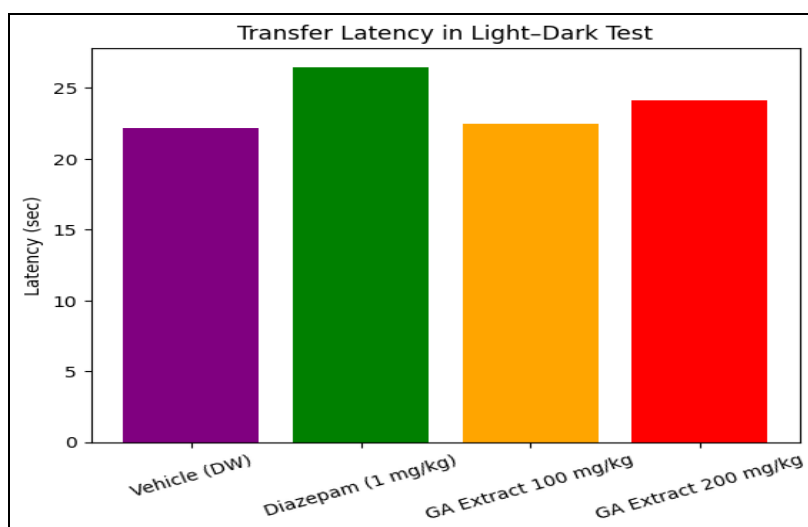


Figure 5.13: Transfer Latency in Light–Dark Test.

Average Percentage Results from Light–Dark Box Test

The anxiolytic activity of the ethanolic extract of *Gmelina arborea* was further assessed using the Light–Dark Box (LDB) model, and the average percentage values for time spent in the dark zone, time spent in the light zone, and the number of crossings were calculated. These parameters help determine the exploratory behavior and anxiety level of the experimental animals.

Table 5.19: Average Percentage Readings from Light–Dark Test.

Treatment	% Time spent in dark zone (sec)	% Time spent in light zone (sec)	% No. of crossing
Group I Vehicle (DW)	94.08	51.62	6.40
Group II Standard Drug Diazepam (1 mg/kg)	43.10	94	15.92
Group III <i>Gmelina arborea</i> Extract (Low Dose 100 mg/kg)	78.96	65.27	9.85
Group IV <i>Gmelina arborea</i> Extract (High Dose 200 mg/kg)	78.32	70.52	11.08

The vehicle-treated control group exhibited typical anxiety-related behavior, as indicated by the highest percentage of time spent in the dark zone (94.08%) and the lowest percentage of crossings (6.40%). Rodents naturally prefer darker environments when experiencing anxiety, which explains the increased time spent in the dark compartment.

The standard drug diazepam (1 mg/kg) produced a significant anxiolytic effect. Animals treated with diazepam showed a considerable decrease in the time spent in the dark zone (43.10%) and a substantial increase in the time spent in the light zone (94%). Additionally, the percentage of crossings increased markedly to 15.92%, indicating enhanced exploratory activity and reduced anxiety levels.

Treatment with *Gmelina arborea* extract at the low dose (100 mg/kg) resulted in a moderate anxiolytic response. The animals showed a reduction in time spent in the dark zone (78.96%) and an increase in time spent in the light zone (65.27%) compared with the control group. The percentage of crossings also increased to 9.85%, indicating improved exploratory behavior.

Similarly, the higher dose of *Gmelina arborea* extract (200 mg/kg) produced a stronger anxiolytic effect than the low dose. The time spent in the light zone increased to 70.52%,

while the percentage of crossings increased to 11.08%, suggesting enhanced locomotor and exploratory activity. These findings indicate a dose-dependent anxiolytic effect of the plant extract.

Overall, the results demonstrate that the ethanolic extract of *Gmelina arborea* reduces anxiety-like behavior in experimental animals, as evidenced by increased exploration of the light compartment and increased crossing frequency in the Light–Dark Box model.

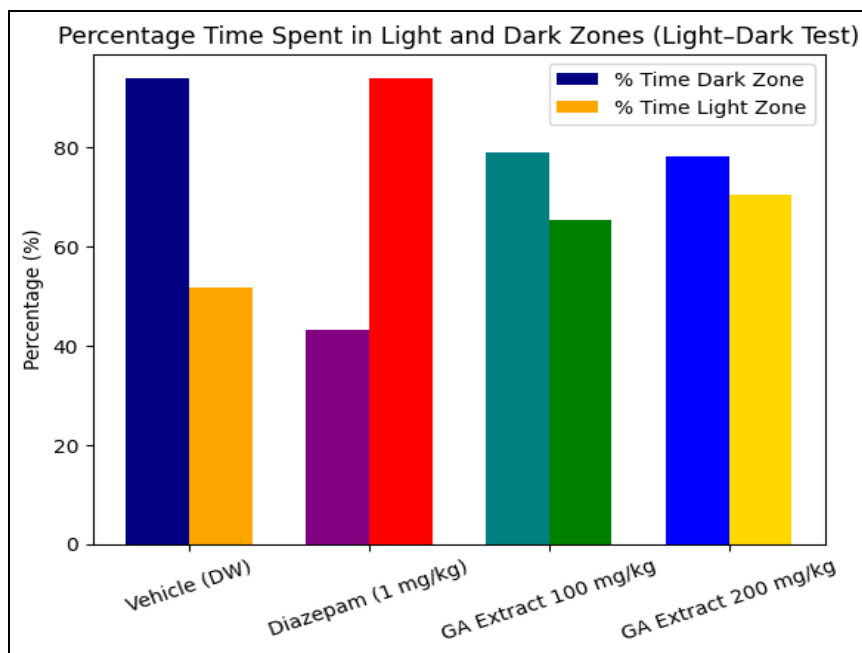


Figure 5.14: Percentage Time Spent in Light and Dark Zones.

ACKNOWLEDGEMENT

I express my sincere gratitude to Dr. Karan B. More for his valuable guidance, constant support, and encouragement throughout the completion of this research project entitled “Anxiolytic Potential of *Gmelina Arborea* Extract Using Elevated Plus Maze and Light -Dark Models in Experimental Animals”. His expertise and insightful suggestions greatly contributed to the successful of this study.

I would also like to extend my heartfelt thanks to Dr. Vikas Rajurkar, Principal of Dr. Vedprakash Patil Pharmacy College, for providing the necessary facilities and a conducive environment for carrying out this research work.

I am grateful to all the teaching and non teaching staff members of the college for their support and cooperation during the course of this study.

Lastly, I express my deep appreciation to my family and friends for their continuous encouragement, understanding, and moral support.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 1999; 12(4): 564-582.
2. Rates SMK. Plants as source of drugs. *Toxicon.*, 2001; 39(5): 603-613.
3. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod.* 2007; 70(3): 461-477.
4. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta.* 2013; 1830(6): 3670-3695.
5. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect.* 2001; 109(1): 69-75.
6. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014; 4: 177.
7. World Health Organization. WHO traditional medicine strategy 2014–2023. Geneva: WHO; 2013.
8. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med.*, 2006; 27(1): 1-93.
9. Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis.* 3rd ed. London: Chapman and Hall; 1998.
10. Trease GE, Evans WC. *Pharmacognosy.* 16th ed. London: Saunders Elsevier; 2009.
11. Evans WC. *Trease and Evans pharmacognosy.* 16th ed. Edinburgh: Elsevier; 2009.
12. Bruneton J. *Pharmacognosy, phytochemistry, medicinal plants.* Paris: Lavoisier Publishing; 1999.
13. Sofowora A. *Medicinal plants and traditional medicine in Africa.* 3rd ed. Ibadan: Spectrum Books; 2008.
14. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* 50th ed. Pune: Nirali Prakashan; 2014.
15. Kumar V, Abbas AK, Aster JC. *Robbins basic pathology.* 10th ed. Philadelphia: Elsevier; 2018.
16. Rang HP, Dale MM, Ritter JM, Flower RJ. *Rang and Dale's pharmacology.* 8th ed. London: Elsevier Churchill Livingstone; 2016.

17. Katzung BG, Trevor AJ. Basic and clinical pharmacology. 14th ed. New York: McGraw-Hill Education; 2018.
18. Stahl SM. Essential psychopharmacology: neuroscientific basis and practical applications. 4th ed. Cambridge: Cambridge University Press; 2013.
19. Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, Davidson JR, et al. Evidence-based pharmacological treatment of anxiety disorders. *Int J Neuropsychopharmacol.* 2014; 17(2): 179-192.
20. Bandelow B, Michaelis S. Epidemiology of anxiety disorders in the 21st century. *Dialogues Clin. Neurosci.*, 2015; 17(3): 327-335.
21. Stein MB, Sareen J. Clinical practice: generalized anxiety disorder. *N. Engl. J. Med.*, 2015; 373(21): 2059-2068.
22. Nutt DJ, Malizia AL. New insights into the role of the GABA-A-benzodiazepine receptor in psychiatric disorder. *Br. J., Psychiatry.* 2001; 179: 390-396.
23. Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders. *Psychol. Med.*, 2013; 43(5): 897-910.
24. Ventola CL. The antibiotic resistance crisis. *Pharm. Ther.*, 2015; 40(4): 277-283.
25. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect. Dis.*, 2013; 13(12): 1057-1098.
26. Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.*, 2010; 74(3): 417-433.
27. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO; 2014.
28. World Health Organization. Mental health atlas 2020. Geneva: World Health Organization; 2021.
29. World Health Organization. Depression and other common mental disorders: global health estimates. Geneva: WHO; 2017.
30. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-5). 5th ed. Washington DC: APA; 2013.