



## ANXIOLYTIC AND ANTICONVULSANT EVALUATION STUDIES OF METHANOLIC EXTRACT OF *TECTONA GRANDIS* LINN. BARK

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### Abstract

Anxiety includes worry and apprehension like symptoms. Women experience higher rates of emotional anxiety than men do when they are teenagers. Epilepsy is a group of disorder of brain with common, sudden and paroxysmal cerebral dysrhythmia with convulsions followed by affected consciousness. The name teak refers to the wood of the *Tectona grandis* plant. It is a significant plant because it's used as medicine in many different indigenous systems. Alkaloids, phenolic glycosides, steroids, etc., are just few of the many identified phytoconstituents. The current study was based on the anxiolytic and anticonvulsant evaluation studies of methanolic extract of *Tectona grandis* bark using different parameters in animal model. Drugs were procured from Sigma Aldrich and some chemicals from local sellers. The bark of plant was harvested at MJP University, Bareilly they were identified and authenticated at the herbarium section of the department of plant science, MJP university. The barks were prepared by using Soxhlet and simple maceration methods. After extraction, it was evaluated for phytochemicals like alkaloids, glycosides etc. OECD guidelines 423 was followed for the dose selection of the extract. Anticonvulsant activity was evaluated in Actophotometer using PTZ induced model and anxiolytic activity in elevated plus maze. In results, the extract of *Tectona grandis* demonstrated a marked reduction in convulsion and anxiety in animals when compared with the control group. In conclusion, *Tectona grandis* plant's bark might be used in the prevention and cure of epilepsy and agitation induced anxiety. It suggests to isolate the active principles and mode of action responsible for the pharmacological activity.

**Keywords:** *Tectona grandis*, anti-convulsant, anxiolytic, PTZ, and EPM.

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**INTRODUCTION**

Anxiety includes worry and apprehension like symptoms. You may begin to sweat, experience chest pain and excitement, and notice your heart racing. Women experience higher rates of emotional anxiety than men do when they are teenagers. The prevalence of anxiety disorders in women is 1.5 to 2.0 times that in men [1]. Adolescents between the ages of 13 and 17 have a lower prevalence of anxiety than adults between the ages of 18 and 64, despite the fact that they are more likely to experience anxiety episodes, separation anxiety disorder, and agoraphobia without having a history of panic attacks [2]. That's why primary care providers often miss the mark when it comes to diagnosing and treating anxiety disorders [3]. Over 700,000 people worldwide take their own lives every year. Suicide ranks as the fourth highest cause of death for people aged 15–29 [4].

Epilepsy is a group of disorder of brain with common, sudden and paroxysmal cerebral

dysrhythmia with convulsions followed by affected consciousness.

Depending upon the movements of the body such as clones and tones and psychiatric phenomena various epilepsy are named [5]. However, a focal origin in the brain that caused generation of signals and discharges spread and postical depression of these regions [6].

The name teak refers to the wood of the *Tectona grandis* plant. It is a significant plant because it's used as medicine in many different indigenous systems. Alkaloids, phenolic glycosides, steroids, etc., are just few of the many identified phytoconstituents [7]. Several components of the plant have been studied for their biological effects, which include anti-oxidant, anti-inflammatory, analgesic, hypoglycaemics, wound-healing, and cytotoxic properties [8].



a. Leaves + flowers



b. Stem

**Fig 1. Different parts of *Tectona grandis* plant**

**Chemical constituents**

**Quinones:** Tectoquinone, lapachol, deoxylapachol and its isomer, tectoleafoquinone, anthraquinone - naphthaquinone pigment, anthraquinone - naphthaquinone pigment

**Steroidal compound;** Squalene, polyisoprene— tolyl methyl ether, betulinic acid, tectograndone, monoterpene are steroidal compounds.

Tectoionols-A and Tectoionols-B are apocarotenoids.

**Glycosides;** Glycosides of anthraquinones  
**Phenolic acids;** Tannic acid, gallic acid, ferulic acid, caffeic acid, and ellagic acid are all phenolic acids.

**Flavonoids;** Rutin and Quercitin from *Tectonagrandis* are flavonoids [9].

<b>Kingdom</b>	Plantae
<b>Super division</b>	Spermatophyta – Seed plant
<b>Division</b>	Magnoliophyta – Flowering plant
<b>Class</b>	Magnoliopsida – Dicotyledons
<b>Subclass</b>	Asteridae
<b>Order</b>	Lamiales
<b>Family</b>	Verbenaceae
<b>Genus</b>	<i>Tectona</i>
<b>Species</b>	<i>grandis</i>

The current study was based on the anxiolytic and anticonvulsant evaluation studies of methanolic extract of *Tectona grandis* bark using different parameters in animal model.

## MATERIAL AND METHOD

### Experimental requirements

Standard drug Diazepam and was obtained from "Sun Pharmaceutical, Gujarat". HCL, KOH, Iodine, Nitric acid, H<sub>2</sub>SO<sub>4</sub>, Fehling solution A & B, Potassium permanganate, n-hexane, Formic acid, NaOH, CuSO<sub>4</sub>, obtained from Merk specialties' Private Limited, Mumbai, Ethanol obtained, from Geetraj Corporation & chemical laboratory, Acetic acid, Ethyl acetate, Picric acid, Chloroform, Potassium mercuric iodide, Potassium dihydrogen orthophosphate, obtained from Fischer Scientific (Qualigens fine chemicals, Mumbai). Biuret reagent obtained from "Central drug house" (CDH) delhi, alpha-naphthol, Millons reagent, Acetone, Formaldehyde, obtained from Fizmerk India Chemicals.

### Collection and authentication of plant

The bark of plant was harvested at MJP University, Bareilly they were identified and authenticated at the herbarium section of the department of plant science, MJP university.

### Extraction

The barks were prepared by using Soxhlet and simple maceration methods, respectively. The alcoholic extra alcoholic and aqueous extract of dried powder (500gm) of the CT was concentrated to dryness under reduced pressure and controlled temperature (48C-50C) with a vapour. The extract was dried in order to produce a dark brown solid extract. The dark brown extract was then subjected to various qualitative phytochemical components. These extracts were used for further biological investigation [10].



**Fig 2. Extraction process through Soxhlet apparatus.**

### Physicochemical parameters

The below physicochemical parameters were taken from guidelines of WHO & Khandelwal, (2002) [11].

### Foreign matter

For this we have to take about 3-5gm of plant part of each extract, and this material have to be aligned in a clean place and then separated into thin form. Inspection has to be done with the help mechanically means or using magnification glass up to 6X. If any foreign material is present then it has to be separated out from the sample and recalculate the sample weight.

### Moisture content

For better standardization of the products, we perform Moisture content; In this method we weight the sample before heating and after placing in the hot air oven and recalculate its weight, when the sample is placed in the oven water is evaporated and we calculate difference in weight with water and loss in water from the content. This method is known as thermo-gravimetric approach.

Moisture content % =  $\frac{X-Y}{W} \times 100$

### Calculate "Alcohol soluble extractive value" (ASE).

% of A.S.E =  $\frac{\text{wt. of TSG/CCS residue extract}}{\text{wt. of TSG/CCS drug}} \times 100$

### Calculate. "Water soluble extractive value" (WSEV)

% of W.S.E.V =  $\frac{\text{wt. of TSG/CCS residue extract}}{\text{wt. of TSG/CCS drug}} \times 100$

### Total Ash value

Total ash% =  $100 \times \frac{(Z-X)}{Y}$  % of A.I.A.V =  $\frac{\text{Final weight (b)} - \text{final weight (a/w)}}{\text{Final weight (b)} - \text{final weight (a/w)}} \times 100$

### Analysis of Fluorescence

Bark sample in the form of powder was taken and few amounts of different chemicals on the glass slide were exposed at 254 and 366nm UV radiation colour then intensity of luminescence was measured.

### Phytochemical analysis

#### Examination for alkaloids

##### Test with Hager's reagents:

Hager's reagent commonly known as saturated solution of picronic acid solution + drug extract was mixed, which gives yellow precipitate.

**Test for Wagner's reagents:**

Wagner's reagent commonly known as Iodine potassium iodide + drug extract was mixed, which gives yellow precipitates, confirms the presence of alkaloids.

**Evaluation of Carbohydrate**

**Fehling's test:**

2 ml of extract were hydrolyzed with dilute HCl and neutralized with alkali and heated with Fehling solution A & B, formation of red ppt indicates the presence of reducing sugar.

**Iodine test:**

2ml of extract were treated with 5 drop of iodine solution, gives blue color indicates the positive test.

**Benedict's test:**

Solution of drug extract with Benedict's reagent was taken in test tube, then this resulting solution is subjected to heat which gives brown ppt. shows the presence of carbohydrate in the extract.

**Test for Glycoside**

**Baljet's Test:**

With the help of Baljet reagent i.e., picric acid solution, add it into test tube about 2ml and add drug extract and after sometimes it develops orange color.

**Legal's Test:**

In a test tube we had taken drug extract and then add 40 ml of both sodium nitroprusside and pyridine which turns pink colour into red colour.

**Keller-Killiani's Test:**

In a test tube take about 40% of FeSO<sub>4</sub> solution in (CH<sub>3</sub>COOH) and add few ml conc. H<sub>2</sub>SO<sub>4</sub> and then add drug extract, resulting solution turns blue which is assay for presence of glycoside moiety.

**Assay of Protein & Various Amino acids**

**Ninhydrin Test:**

In a test tube we had taken drug extract and ninhydrin reagent and heat the resulting solution for 2 min which showed a distinguished color of purple, proves the active moiety of proteins.

**Xantho-protein Assay:**

In a test tube we had taken drug extract and add 40ml of sulphuric acid which gives white ppt. Then we warm above mentioned solution in which white ppt convert it into yellow ppt, then we add ammonium hydroxide which convert yellow colour into orange colour.(17)

**Test for Phenolic Compound and Tannins.**

**Ferric chloride test:**

Test extract were treated with 4 drop of alcoholic ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

4 ml of extract and shake well wait for 15 min, foam formation indicates positive test.

**Test for Steroids.**

**Liebermann- Burchard's Test:**

In a test tube we had taken drug extract, and then we add few ml of chloroform solution + few ml of conc. Sulphuric acid and few drops of dil. Acetic acid + 3 ml of C<sub>4</sub>H<sub>6</sub>O<sub>3</sub>, which gives bluish-green colour which confirms the presence of steroids.

**Test for flavonoids:**

**From hydrolytic Sodium hydroxide solution**

In a test tube we had taken drug extract, then we add hydrolytic NaOH solution, showing yellow color prove the presence of flavonoids.

**Alkaline reagent test:**

Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of flavonoid.

**Zn Test:**

2 ml extract were treated with Zn dust and conc. HCl development of red colour indicates presence of flavonoids.

**Examination for Saponins**

**Froth Test-**

Presence of saponins is detected by taking drug extract along with water into test tube and it was vigorously shaken which shows appearance of froth which is then stabilized after 405 min confirms the presence of saponins.

**Thin Layer Chromatography**

According to guidelines of WHO Geneva 2002, the thin layer chromatography plate was prepared, which is further activated in order to get precise result [12].

**Preparation of sample:**

Drug extract about 0.5gm was dissolved in water, which is further mixed and shaken about 5-25 min. Insoluble part of mixture was removed by filtration and centrifugation means.

**Adsorbent Preparation:**

Silica gel G slurry was prepared by weighing 400gm of silica gel G which was dissolved in water and with the help of spreading device, it was gently spread into TLC plates and forming layer of about

0.20-0.25mm thickness. For activation of plates it was kept in hot air at 100 degree oven about 20min. Uniformly with the help of transmitted light coating material was inspected in and the texture in reflected light.

#### Saturation of TLC chamber:

Suitable amount of mobile phase added into saturation chamber and top of the area was covered with the help of petri-dish and filter paper in order to get uniformly saturate chamber. About 40hr it was kept without any disturbance at room temperature, the relative humidity was 540-640% during the experimental separations.

#### Application of compounds:

With the help of micropipette placed the spots of drug extract solution on to the developing TLC plate at 15mm at the edge of the plate.

#### Mobile Phase Preparation

Different concentration of mobile phase were developed for effective separation of its constituents, various mobile phase include-  $\text{CHCl}_3 + \text{CH}_3\text{OH} + \text{H}_2\text{O}$  (5:40:405),  $\text{C}_4\text{H}_8\text{O} + \text{C}_3\text{H}_6\text{O} + \text{H}_2\text{O}$  (405:5:3),  $\text{C}_4\text{H}_9\text{OH} + \text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O}$  (40:4040:404),  $\text{C}_4\text{H}_9\text{OH} + \text{CH}_3\text{COOH} + \text{H}_2\text{O}$  (402:3:5) and spots were identified under daylight, then under shorter-wave length and longer-wave length UV and it was determined various spots by help of n-butanol : acetic acid : Formic: water (402:3:5) mobile phase.

#### Chromatogram Development:

The plate containing spot were dry at room temperature, then the chromatographic plate was kept at 90 degrees into chromatographic (saturation) chamber and it was ensured that the spot was held at top of the surface of the running chamber, during procedure it was closed. All this procedure had to done at room temperature.

#### Chromatogram Observation:

Various spot-on TLC plate was seen under the exposure of UV light having wavelength about 200-400nm. The Retention factor was calculated using the below formula.

$$R_f = a/b$$

#### Animal preparation

From CDRI (Central drug research institute), animal house we get sixty young Wistar albino rats having weight ranges in between 150-200 gm for our research purpose. For storage purpose these animals are kept in cage, which is made up of polypropylene having hygienic and standard conditions in which there is 12hr

dark condition and 12hr light condition, maintained at  $24 \pm 2$  conditions is maintained. Same diet is given during research protocol i.e before and after the experiment, and the diet was pellet and libitum ad water. For better accommodation the experimental animals were kept in isolation specified area in animal house at-least before the 14 days prior to start the protocol for the experiment, which is approved by I.A.E.C having. Registration-Number is IAEC/DECEMBER-20210/22) [13].

#### Dose regimen

With the help of O.E.C.D guidelines No-423, LD50 is calculated in trail animals for the better dose determinations. For finding of suitable dose of extracts we administered by means of orally with help of oral-gavage having dose in range of (5,50,300,2000 mg/kg) body weight of trials animals. For this we take a group of three- animals (usually female) and give dose. For calculating acute toxicity of the test substance of the extract, we need to go through about 2-4 steps, which is depend on mortality / morbidity of trial animals. Observation period for such study required at-least 15 days after dosing. For assessment of acute toxicity. The trial animals, received 2000mg/kg of extract in every group of six trial animals and observation period needs at a interval of 1, 2, 4, 8, 402 and 24 hrs [14].

#### TG extract preparation of dose:

Dose ranges between 100 and 200mg/kg body wt., of alcoholic extract of TG were suspended in unionized water. And administered orally with the help of oral gauge to "Wistar albino rats"

#### Epileptic induced in Experimental Animals

About 40mg/kg. body. wt. Dose of PTZ dissolved in saline solutions and this dose administered by I.P route for better efficacy. For development of epileptic in rats it takes nearly one day [15].

#### Group design

Experimental animals were classified as randomly into five groups and total 30 experimental animals were taken in which, each group receive six animals-

GROUP	TREATMENT
Control	Normal Saline (2ml, p.o)
Standard	Diazepam (2mg/kg i.p.)
Test I	Plant extract (100mg/kg i.p.)
Test II	Plant extract (200mg/kg i.p.)

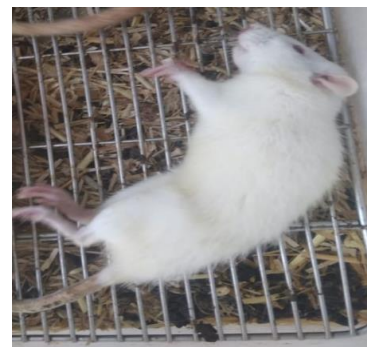
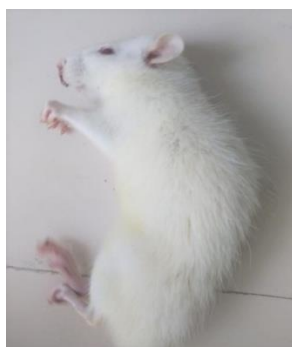
### Animal and their treatment for Anti-convulsant Activity

Experimental animals were classified as randomly into five groups and total 30 experimental animals were taken in which, each group receive six animals;

Inducing agent: Animals were given inducing agent i.e., Nicotine 2mg/kg b.wt.

GROUP	TREATMENT
Control	Normal Saline (2ml, p.o.)
Standard	Sodium Valproate (1-5mg/kg, i.p.)
Test I	Plant extract (100mg/kg, i.p.)
Test II	Plant extract (200mg/kg, i.p.)

### SCREENING PARAMETERS



(i) Facial, vibrissal and forelimb clonus (ii) Sporadic forelimb clonus (iii) Clonus/or wet dog shake behaviour  
**Fig 3. Pictures showing the induction of seizure in Wistar albino rats with different posture according to Racine seizure scale during Nicotine induced kindling model of epilepsy Record the convulsion behaviour of rats for 30 minutes immediately after the injection of inducing agent. It is clear from the picture that from the first dose of nicotine to 10<sup>th</sup> dose, rat showed different behaviour response which can be easily assessed by Racine seizure scale.**

### Elevated Plus Maze (EPM)

EPM (Elevated plus maze) behavioural model principally works as giving incentive from the outside environment not as inside ones, basically used as to examine cognition impact in rats/ mice. EPM consist of two arms i.e., one is open arm and other one is closed arm. There are basically two open arms and two closed arms. Dimension of open arm and closed arm are basically same dimension i.e., 48cm x 10cm. Some extra modification are seen in closed arms i.e., it consist an open roof with 40cm high walls. To measure anxiety level in experimental animal the EPM was elevated up to 50cm height [17].

Basically, two parameters are used in EPM are Transfer Latency and Memory retention. In Transfer latency we put animal at the edge of open arm and placing of the mouse is in the direction of far from the mean of the platform. To measure "Transfer latency" we note down the time spent by the rat to enter one of its closed appendix with all its four legs. In case if rats did not enter into the closed arm within 3 minutes, then it is delicately

### Assessment of locomotor activity

For locomotor activity, rats were individually placed in the centre of an open box (80x80x40cm) with the floor divided into 16 squares. The behaviours were video recorded, and the number of squares crossed with all four paws (ambulation) in the centre zone or near to the wall during 5 minutes were recorded and analysed [16].

### Induction of seizure

Induction of seizure was done in Wistar albino rats of average body weight (150-200g). The disease was induced through chemical induced model by administration of Nicotine at doses (1-5mg/kg., b. wt).

driven into one of the secured arms inside 180 sec, and in this case TL is assigned as 180sec. At that point of following 15 sec., rodent was permitted for investigate the labyrinth before returning it to its home cage. Memory maintenance is inspected 24 h after the principal day preliminary onthe subsequent day. Between every meeting, the labyrinth was painstakingly cleaned with 30% ethanol to expel any olfactory signs.

## RESULTS AND DISCUSSION

### Analysis of physicochemical parameters

*Tectona grandis*, are mentioning it below table:

**Table 1. Physicochemical Parameters of coarse Powder consist of:**

Parameters	% (w/w)
Loss on drying at 105°C (% w/w)	7.0
Complete (Ash value)	4.6
Acid insoluble (ash value)	7.4
Polar- water soluble (extractive value)	27
Alcohol-soluble-extractive value	4.3
Swelling index (% w/w)	40.7
Foaming index (% w/w)	6.3

**Analysis of fluorescence**

The result of Fluorescence of crude powder of *Tectona grandis* as. mentioned it below.

**Table 2. Fluorescence evaluation of crude coarse powder *Tectona grandis* consist of:**

Reagents	UV Light (254nm)	UV Light (366n)	Visible light
Coarse Powder	Light Yellowish Brown	Yellowish Brown	Yellowish brown
Treated with water	Light Yellowish Brown	Light Brown	Dark Yellowish brown
50% Sulphuric acid	Light Brownish	Black Brown	Brown
Conc. HCl	Brown	Reddish Brown	Darkish Brown
Chloroform	Yellow	Yellowish Red	Orange
Conc. Nitric acid	Greenish Yellow	Reddish brown	Brownish orange

**PHYTOCHEMICAL ANALYSIS****Table 3. The result of phytochemical analysis of crude powder of defatted of *Tectona grandis* species extract**

Test Performed	Ethanollic extract	Aqueous extract
Alkaloids		
Mayer's Reag.	+	++
Dragendarff's Reag.	+	+
Wagner's Reag.	+	+
Hager's Reag.	+	++
Saponins		
foam investigation	+	+
Steroids		
Salkawoski Test	++	++
Leiberman's Reagent	+	++
Carbohydrates		
Molisch's Test	+	+
Fehling's assessment	++	++
Anthraquinone Glycosides		
Borntrager's Test	++	+
Cardiac Glycosides		
Keller killiani Test	-	+
Tannins		
Pb(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> Solution	-	+
FeCl <sub>3</sub> Solution	+	++
Proteins		
Xanthoprotein assay	-	+
Biuret assay	-	-
Flavonoids Test		
Shinoda assay	++	+++
Cyanogenic Glycosides	--	--

**THIN LAYER CHROMATOGRAPHY**

For efficient departure of different constituents present in the sample, various variety of mobile phase of variant composition had been used, which includes solvent systems like - CHCl<sub>3</sub>+CH<sub>3</sub>OH+H<sub>2</sub>O (5:10:15), C<sub>4</sub>H<sub>8</sub>O+C<sub>3</sub>H<sub>6</sub>O+ H<sub>2</sub>O (15:5:3), C<sub>4</sub>H<sub>9</sub>OH+C<sub>2</sub>H<sub>5</sub>OH+H<sub>2</sub>O(10:10:14),C<sub>4</sub>H<sub>9</sub>OH+CH<sub>3</sub>COOH+H<sub>2</sub>O (12 : 3 : 5) and spots were identified under daylight, then under shorter-wave length and longer-wave length and it was determined various spots by help of n-butanol : acetic acid : Formic:

water (12 : 3 : 5) mobile phase.

**Table 4. Showing different *R<sub>f</sub>* value for Extract are given below**

Solvents system	<i>R<sub>f</sub></i>
CHCl <sub>3</sub> +CH <sub>3</sub> OH+H <sub>2</sub> O	0.25
C <sub>4</sub> H <sub>8</sub> O+C <sub>3</sub> H <sub>6</sub> O+H <sub>2</sub> O	0.55
C <sub>4</sub> H <sub>9</sub> OH+C <sub>2</sub> H <sub>5</sub> OH+H <sub>2</sub> O	0.43
C <sub>4</sub> H <sub>9</sub> OH+CH <sub>3</sub> COOH+H <sub>2</sub> O	0.65



a) Rf =0.55b)



b) Rf =0.65

**Fig 4.** Showing various spot of chemicals present in TLC- plate of plants extract.i.e *Tectona grandis*

### Acute oral toxicity study

For determining of oral acute toxicity study we take a dose of plant part at various dose i.e. 10mg/Kg, 100mg/Kg, 500mg/Kg and 1000 mg/Kg of their body weight for assesment of their toxicity at a specific interval of time, it has been reported that no change in their behaviour and not showing any kind of abnormal behaviour and no mortality was observed during the study.

### Tests for screening anticonvulsant activity

#### *Pentylenetetrazol induced seizure (PTZ)*

The test drugs and (saline for control group) were administered orally and one hour later Pentylenetetrazol 40mg/kg/i.p was given and onset of first jerky movement, onset of Straub's tail, onset of clonic convulsions, onset of tonic flexion, onset of Hind limb tonic extension (HLTE) and reduction in mortality were measured (the blockade of clonic convulsions, or an increase in convulsion latency, indicates an anticonvulsant effect.).

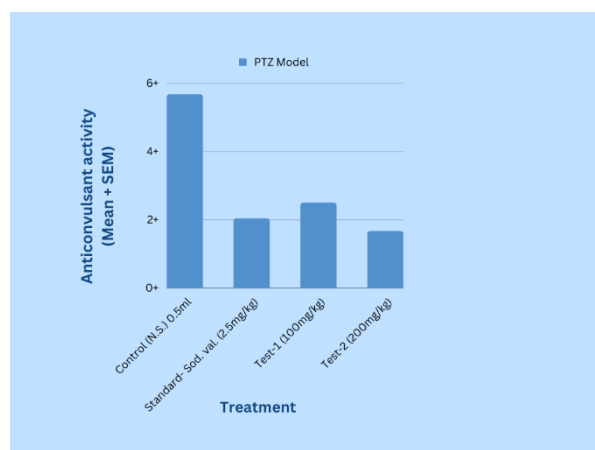
Swiss albino mice Sex: Male Number of animals: 30 mice (6 per group x 6 groups) Dose levels: vehicle-treated control group, and up and down procedure starting from 100mg/kg with an increment of 100mg/kg until 100% protection is achieved or at least three groups treated with different doses of the test compound. Route of administration: Oral 92 Vehicle: 0.2% Dimethylsulfoxide in distilled water Post-treatment time:

1. hour Mice were brought to the laboratory and allowed them to acclimate to the test room for at least 30 min before use.
2. Mice were selected, their weights were recorded, and unique identifying mark on each animal was placed. Mice were returned to their holding cages to await dosing. Care was taken not to mix mice that were housed in different home cages to avoid untoward behavioural reactions (e.g., aggression)
3. 0.5ml Vehicle (0.2 % Dimethyl sulfoxide) was administered to each mouse of the control group. Administration was staggered (1- to 3-min intervals) to maintain the same time

between compound administration and testing for each animal

4. Mice were returned to their holding cages as soon as they are treated with the test compound or vehicle
5. after the post-treatment time (One hour) has elapsed, by grasping the mouse firmly by the nape of the neck, pentylenetetrazol at 40mg/kg/body weight intraperitoneally (i.p) was administered
6. Following PTZ injection, mouse was immediately placed into a separate observation cage to monitor the course of the seizure.
7. The blockade of clonic convulsions, or an increase in convulsion latency, (indicates an anticonvulsant effect) was recorded.
8. Steps 8 to 12 repeated for all of the mice in the group in the order they were dosed.
9. Entire protocol repeated for each treatment group.
10. Calculate the effect of *Tectona grandis* bark extract on anticonvulsant activity.

Treatment	Anticonvulsant activity (Mean + SEM)
Control (N.S.) 0.5ml	5.67+2.10
Standard- Sod. val. (2.5mg/kg)	2.04+1.30
Test-1 (100mg/kg)	2.50+1.87
Test-2 (200mg/kg)	1.67+1.86



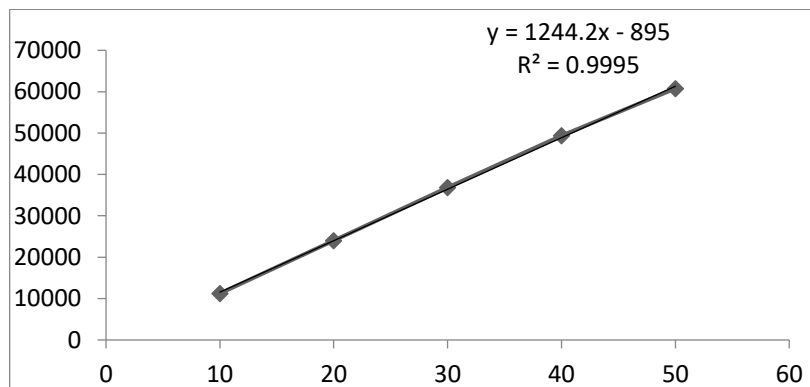
**Fig 5.** Anticonvulsant activity in PTZ model



**Estimation of Gamma-amino butyric acid (GABA) through High Performance Liquid Chromatography (HPLC)**

Induction of epilepsy through the chemical inducing agent (nicotine) at a dose (35-55 mg/kg) i.p. was done and treated by fixed dose combination of various dose.

Micro gram/ml	Calibration Curve (Area)
10	11234
20	23985
30	36816
40	49371
50	60752



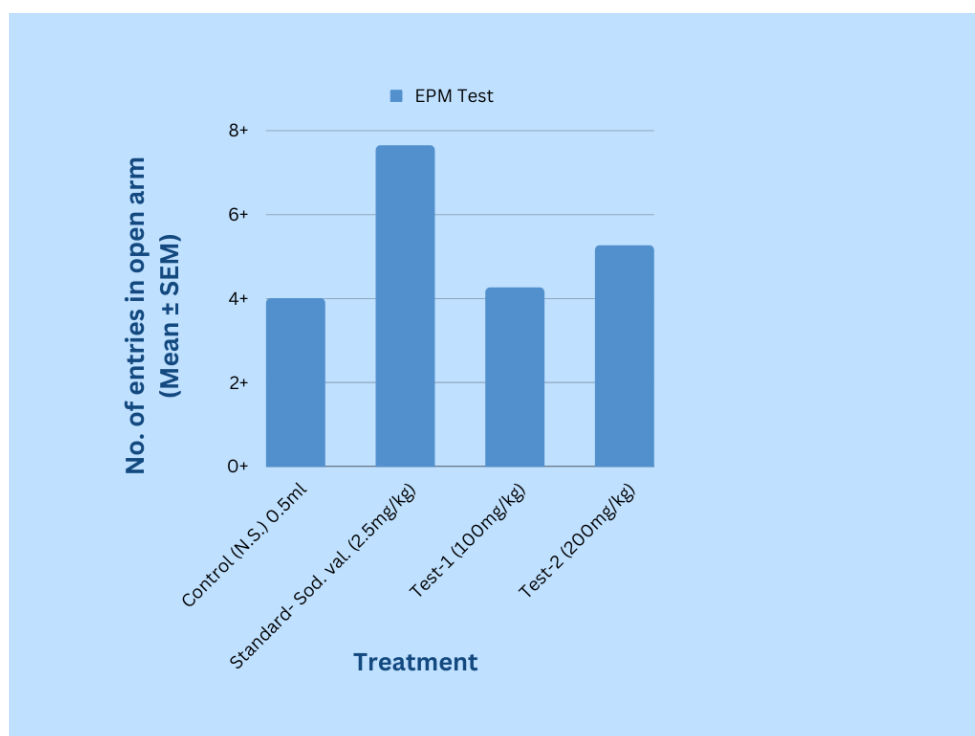
**Fig 5. Calibration Curve of GABA**

Evaluate of Anxiolytic and Anticonvulsant evaluation studies of methanolic extract of *Tectona grandis* Linn bark.

**Table 5. Effects of. Alcoholic extract of *Tectona grandis* on no. of entries in open Arm.**

Treatment	No. of entries in open arm (Mean ± SEM)
Control (N.S.) 0.5ml	4.0+ 0.39
Standard- Sod. val. (2.5mg/kg)	7.64+ 0.70
Test-1 (100mg/kg)	4.26+0.38
Test-2 (200mg/kg)	5.26+ 0.24

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)

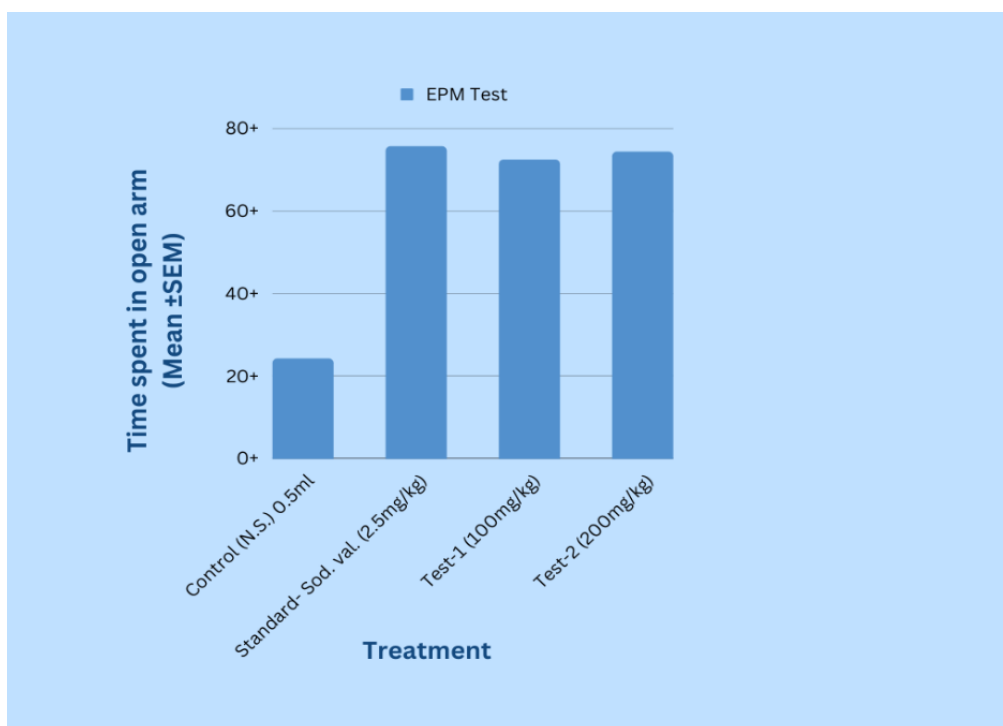


**Fig 6. No. of entries in open arm in EPZ test**

**Table 6. Effect of *Tectona grandis* on time spent in open arms:**

Treatment	Time spent in open arm (Mean $\pm$ SEM)
Control (saline water)0.5ml	24.22+0.30
Standard (Diazepam)2mg/kg	75.6+0.71
Test-1LTGB 100mg/kg	72.4+0.76
Test-2 HTGB 200mg/kg	74.3+0.80

Values were given in Mean  $\pm$  S.E.M. and found statistically significant at  $P < 0.05$ , compared to control (n=6)

**Fig 7. Time spent in open arm in EPM test**

For standardization of alcoholic extract of plant materials include various parameters had been performed like presence of unwanted substance, and also determine its various extractive value, physiochemical parameters and pharmacognostical parameters. Also find its various index include swelling and foaming index. Parameter like loss of drying reveals the presence of moisture content in the drugs. Presence of various compounds in the plant extracts like alkaloids, glycoside, polyphenolic compounds, flavonoids, carbohydrates, steroids, amino-acids and amygdalin shows its presence of its bioactive chemical constituents which helps in its identification, determination and shows its potential to treat various disease. Fluorescence analysis carried for checkout the genuineness of the powder of the crude drug. The crude drug under fluorescence analysis showed different colour with different no. of reagent which confirmed various type of constituent present in the drug. Preliminary

phytochemical tests determined the presence of alkaloids, carbohydrate, amino acids, proteins, amygdalin and flavonoids. Some researchers have reported Phenolic comp. flavonoids & steroidal glycosides act as active ingredients in the treatment of epilepsy. During research work it had been observed that antioxidant activity of plant extract of seeds extract may be attributed to the presence of above bioactive ingredients & their advantageous combined effect.

In results, the extract of *Tectona grandis* demonstrated a marked reduction in convulsion and anxiety in animals when compared with the control group.

## CONCLUSION

Based on above discussion, it may be concluded that plant extract potentiates GABA<sub>A</sub> currents in a distinct way. The knowledge of this biological effect can be used manage the neuronal hyper

excitability and increase the seizure threshold caused by imbalance between GABA and Glutamate. Along with antiepileptic activity plant material also reduced oxidative stress and thus exert an antioxidant activity. The anti-anxiety and anti-convulsant activity study for the extract of bark of *Tectona Grandis* will be performed and this study may provide experimental data in support of effective of anti-anxiety and anti-convulsant activity particular portions of the plan.

In conclusion, *Tectona grandis* plant's bark might be used in the prevention and cure of epilepsy and agitation induced anxiety. It suggests to isolate the active principles and mode of action responsible for the pharmacological activity.

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#### CONFLICT OF INTEREST

Authors declared for none conflict of interest.

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