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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *TERMINALIA CATAPPA LINN.* LEAVES EXTRACTS FOR ANTIANXIETY ACTIVITY

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ABSTRACT

The present study investigate physicochemical characterization, antioxidant and Antianxiety activity of extracts from *Terminalia catappa* leaves collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using Acetone and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed. The presence of phytoconstituents such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of acetone and methanol leaves extracts. Maximum percentage inhibition i.e. 80.97% was shown by methanolic extract at concentration of 150 µg/ml and was compared with Ascorbic acid as reference standard. The *In-Vivo* Antianxiety activity of *Terminalia catappa* leaves was evaluated by Elevated plus maze and Light and Dark model in rats using Diazepam as a standard. Both the extracts at 200mg/kg concentration showed significant to highly significant number of entries & time spent in P zone (from $P < 0.05$ to $P < 0.001$). The study reveals that *Terminalia catappa* leaves extracts possess Antianxiety activity and this might be due to flavonoids. Phenolic compound, steroid and proteins present in extract.

Keywords: *Terminalia catappa,* Acetone and Methanolic extract, Phytochemical screening, Antioxidant effect, Antianxiety activity.

I. INTRODUCTION:

Anxiety disorders are the most prevalent psychiatric disorders. There is a high comorbidity between anxiety (especially generalized anxiety disorders or panic disorders) and depressive disorders or between anxiety disorders, which renders treatment more complex. Current guidelines do not recommend benzodiazepines as first-line treatments due to their potential side effects. Selective serotonin reuptake inhibitors and selective serotonin norepinephrine reuptake inhibitors are recommended as first-line treatments. Psychotherapy, in association with pharmacotherapy, is associated with better efficacy. Finally, a bio-psycho-social model is hypothesized in anxiety disorders. Anxiety disorders are the most prevalent psychiatric disorders (with a current worldwide prevalence of 7.3% [4.8%- 10.9%]

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Among them, specific phobias are the most common; with a prevalence of 10.3%, then panic disorder (with or without agoraphobia) is the next most common with a prevalence of 6.0%, followed by social phobia (2.7%) and generalized anxiety disorder (2.2%). Evidence is lacking as to whether these disorders have become more prevalent in recent decades. Generally speaking, women are more prone to develop emotional disorders with an onset at adolescence; they are 1.5 to 2 times more likely than men to have an anxiety disorder [1, 2].

The Tropical Almond Tree chiefly known as *Terminalia Catappa*. It is a large deciduous tree. It grows up to 90 feet tall. The leaves turn red to yellow before falling. All parts of the plant like leaves, kerel, bark, root, wood and fruit are used for medicinal purposes. The leaves and Bark extract of the tree possess Anti Carcinogenic, Anti HIV, Hepatoprotective, Anti Diabetic and Liver regenerating effects. The leaves are Anti Sickling in nature it is beneficial for Liver detoxification and supports the immune System. The leaves contain several flavonoids (such as kaempferol or quercetin), several tannins (such as punicalin, punicalagin or tercatin), saponines and phytosterols. Due to this chemical richness, the leaves (and the bark) are used in different herbal medicines for various purposes. Keeping the leaves in an aquarium may lower the pH and heavy-metal content of the water. The phytochemicals of *T. catappa* leaf contain chebulagic acid, corilagin, kaempferol, punicalagin, punicalin, quercetin, tercatain, tergallagin, terflavin A, and terflavinB [3]. The main of the article is phytochemical and pharmacological evaluation of *terminalia catappa* linn. leaves extracts for antianxiety activity.

II. MATERIAL AND METHODS:

1. Collection, identification and authentication of plant material

Fresh leaves were collected in the month of October from local region of Nanded district and the plant was authenticated by Dr.S. S. Bodke, Associate Professer & Head of Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded. A voucher specimen of plant was preserved in the herbarium (NPC/M. Pharm/herbarium/2019-20/H-4) for further reference. Collection, authentication, identification, processing and storage have been done according to standard procedure for the plant material.

2. Processing of crude drug:

The collected leaves were dried under shade, segregated and further crushed to coarse powder by mechanical grinder and the powder was passed through No. 14 sieve.

3. Preparation of Extracts [4]:

Three extracts of *Terminalia catappa* leaves powder were prepared

1. Pet ether
2. Acetone extract
3. Methanol extract

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\%\ \text{yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

A. Preparation of Acetone extract

Acetone extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 300 gm of the powdered and 1000 ml Acetone was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

B. Preparation of Methanol extract

Methanolic extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 150gm. of the powdered and1000 ml Methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

III. PHYTOCHEMICAL EVALUATION:

A. CHEMICAL TEST [4].

1. Detection of alkaloids:

Extracts were dissolved individually in dilute HCl and filtered.

Dragendorff's test:

To 2-3 ml Filtrate, add few drops of Dragendorff's reagent. Orange brown Ppt. formed indicates the presence of alkaloids.

Hager's test:

To 2-3 ml Filtrate Hager's reagent Formation of yellow precipitate indicates the presence of alkaloids

Tannic acid test:

Test solution treated with tannic acid solution gives buff colored precipitate the presence of alkaloids.

2. Detection of proteins & amino acid:

Million's test:

Mix 3 ml test solution with 5 ml Million's reagent. White precipitate warm precipitate turns brick red precipitate dissolves giving red colored solution indicates the presence of protein.

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Ninhydrin test:
To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.
Biuret test:
To 3 ml test solution adds 4% NaOH and few drops of 1% Copper sulphate solution. Violet color appears.

3. **Detection of carbohydrates:**
Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates
Molish's test:
Filtrates were treated with 2 drops of alcoholic α - naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.
Barfoed's test:
Mix equal volume of Barfoed's reagents and test solution. Heat for 1-2 min in boiling water bath and cool Red precipitate is observed
Benedict's test:
Filtrates treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
Fehling's test:
Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A &B solutions. Formation of red precipitate indicates the presence of reducing sugars.
Detections of glycosides:
Extracts were hydrolysed with dil.HCl, and then subjected to test for glycosides.
Modified Borntrager's Test:
Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink color in the ammonical layer indicates the presence of anthranol glycosides.

4. **Detection of tannins:**
To 2-3 ml of aqueous or alcoholic extract, add few drops.
5% Ferric chloride test: deep blue – black color
Lade acetate sol. Test: White precipitate

5. **Detection of Flavonoids:**
Lead acetate test:
Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.
Shinoda test:
To dry powder or extract add 5 ml 95% ethanol few drops conc. HCL and .0.5 gm. magnesium turnings. Orange, pink, red to purple color appears. Add t-butyl alcohol before adding the acid to avoid accidents from a Violent reaction and magnesium, only flavones give a deep red to magenta color while flavones and flavones give weak pink to magnetic color is observed.

6. **Detection of phytosterols:**
Salkowski's test:
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

7. **Detection of Saponin:**
Foam test:
Shake the drug extract or dry powder vigorously with water. Persistent foam observed indicates the presence of saponin.

IV. DEVELOPMENT OF TLC FINGERPRINT [4].

1. **Introduction:**
Thin layer chromatography is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid, is allowed to migrate across the surface of the plate by capillary action by gravity or pressure. TLC separation takes place in the open layer with each component having the same total migration time but different migration distance. Numerous fixed adsorbents have been used, including Silica gel, Cellulose, Polyamide, Alumina, Ion exchange and chemically bonded silica gel. Mobile phase consists of a single solvent or a mixture of solvents.
The stationary phase of the TLC is prepared using various techniques such as pouring, dipping and spraying. The prepared plates are allowed for setting (air-drying). This is done to avoid cracks on the surface of adsorbent. After setting the plates are activated by keeping in an oven at 100 to 120^{0}C for one hour. Activation of TLC plates is nothing but removing water/moisture and other substances from the surface of any absorbent, by heating at temperature around 110^{0}C so that adsorbent activity is retained. TLC studies were carried out using various extracts to confirm the presence of different phytoconstituents in the extract.

Analysis
In TLC qualitative analysis of the unknown compound is done by comparing the R_f values. As solutes never travel the full length of the stationary phase in TLC all the R_f value depends on the amount of the stationary phase, the humidity, layer thickness, solvent quality, saturation of chamber,

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development distance, temperature, amount of substance added, and the presence of impurities.

$$R_f = \frac{\text{Distance from origin to the point of maximum intensity}}{\text{Distance from origin to the solvent front}}$$

R_f = Retention factor

2. **Total Phenolic Content [5, 6].**
Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1m) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10 ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCo_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

3. **Total Flavonoids Content [5, 6].**
Total Flavonoid Content was measured by the alluminium trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

4. ***In vitro* anti-oxidant activity [7, 8].**
An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.

2,2 Diphenyl- 1 picryl-hydrazylradical scavenging (DPPH) Activity:

Principle:
The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 1, 1- diphenyl-2-picryl-hydrazyl.

Reagents Required:
1) DPPH
2) Pure Methanol

Preparation of samples and standard solutions:
Accurately weighed 10 mg of Acetone and Methanolic extracts and the standard ascorbic acid and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure:
The reaction mixture (3.0 ml) consists of 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was vortexes and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

The percentage of inhibition can be calculated using the formula:

$$\%\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,
$A_{control}$: Absorbance of control.
A_{test}: Absorbance of test.

V. ANIMAL USED:

For the study *Wistar rats* of either sex, of weight 150-200gm were selected.

Test group:
For the study seven groups of animals were made. Each group was consisted with six rats. **(1613/PO/Re/S/12/CPCSEA).**

Route of administration: Oral route and ip. Route of administration.

Housing Condition:
Animals were housed seven groups in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of six rats

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VI. EVALUATION OF ANTIANXIETY ACTIVITY [9]:

Evaluation of **Antianxiety** activity was done by using following models;

1. Elevated plus maze

Principle:

This model is based on natural behaviour of rodents for open spaces and fear of height. Rodents always tend to avoid the open areas and stay in darker areas, more enclosed spaces. When animal is placed on EPManxious animals spend more time in enclosed arms and non-anxious animals explore and spend more time on open arms. Anxiolytic compounds by decreasing anxiety, increases the open arm exploration time.

Animal Grouping:

Group A - Control: DMSO (0.5% in water)
Group B- Standard: Diazepam (2mg/kg)
Group C - Test Dose-I: TCAE (200 mg/kg)
Group D- Test Dose-II: TCAE (100 mg/kg)
Group E- Test Dose I: TCME (200mg/kg)
Group F- Test Dose II: TCME (100mg/kg)

Fig. 1: Elevated plus maze model

Procedure:

The elevated plus maze consist of two open arms, 35X15X15 cm & two enclosed arms, 35X15X15 cm, that extend from a common central platform; with an open arm roof, arranged so that the two open arms are opposite to each other. The entire maze was elevated to a height of 50 cm above the ground level. The rats were housed in group of six in cages prior to testing in apparatus. During this time the rats were handled by investigator on alternate days to reduce stress. The animals were divided in six groups. Four groups test drug (p.o.) & standard drug (i.p.) were administered 1 hour before testing. After1 hr rat was placed in centre of maze, facing one of enclosed arms. During a period of 5 min the following parameters were observed by using video tracking system; Number of entries in open arm & enclosed arms, time spent in open arm, enclosed arm & centre, total number of arm entries

Evaluation:

Evaluation of antianxiety activity was done by observing the parameters like number of entries in open & enclosed arm, time spent by the rats in open & enclosed arms & comparing these parameters with that of control group. The anxiolytic agents increase the motor activity there by open arm exploratory time.

2) Light-dark method

Principle:

In a two chambered system, where the animals can freely move between a brightly-lit open field and a dark corner, they show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics.
The numbers of crossings between the light and dark sites are recorded.

Animal Grouping:

Group A -Control: DMSO (0.5% in water)
Group B- Standard: Diazepam (2mg/kg)
Group C - Test Dose-I: TCAE (200 mg/kg)
Group D- Test Dose-II: TCAE (100 mg/kg)
Group E- Test Dose -III: TCME (200mg/kg)
Group F- Test Dose –IV: TCME (100mg/kg)

Procedure:

The testing apparatus consists of two compartment chamber (47X27X27 cm) comprising of two-third brightly illuminated area & one-third dark area separated by a wall with a round hole (13 cm long X 5 cm high). A partition containing a opening separate the dark one third from the bright two third of the cage. The animals were treated with test drug (p.o.) & standard drug (i.p.) half hour prior testing. The rats were placed individually in the illuminated part of the cage & the electronic video tracking system was used to automatically count movements through the partitions & clocked the time spent in light & dark compartments.

Evaluation:

The parameters like time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments & transfer latency of rats were evaluated. Anxiolytic agents increase the total loco motor activity.

VII. STATISTICAL ANALYSIS

The data were expressed as mean + standard of mean (SEM).Statistical analysis were performed by one way analysis of variance (ANOVA).

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VIII. RESULTS:

1. Phytochemical tests of *Terminalia catappa* leaves extract:

Table 1: Observations for Phytochemical qualitative analysis

Sr. no	Test for	Pet ether	Acetone	Methanol
1	**Alkaloids**			
	Wagner test	+	+	+
	Hager's test	-	+	+
2	**Proteins**			
	Millon's test	+	+	+
	Xanthoprotein test	+	+	+
	Biuret test	-	-	+
3	**Carbohydrate**			
	Molish's test	+	+	+
	Barfoed's test	-	+	+
	Benedicts test	+	-	+
4	**Glycosides**			
	Borntrager's test	-	+	+
	Keller killani test	+	+	+
5	**Tannins and Phenolic comp.**			
	Ferric chloride test	-	+	+
	Lead acetate sol^{n}test:	+	+	+
	Dil. Nitric acid test	+	-	+
6	**Flavonoids**			
	Alkaline test	+	-	+
	Shinoda test	+	+	+
8	**Steroids**			
	Salwoski test	-	+	+
	Libberman test	-	-	+
9	**Amino acid**			
	Ninhydrin test	+	+	+
	Tyrosin test	+	-	-
	Tryptophan test	-	+	+

(+): present (-): Absent Above observation table shows the presence of phytoconstituents in the extracts. It reveals all three (i.e Pet ether, acetone and methanol) extracts contain carbohydrates, Glycosides, steroids, tannins, flavonoids, Saponins, Amino acid and Proteins.

2. TLC fingerprinting

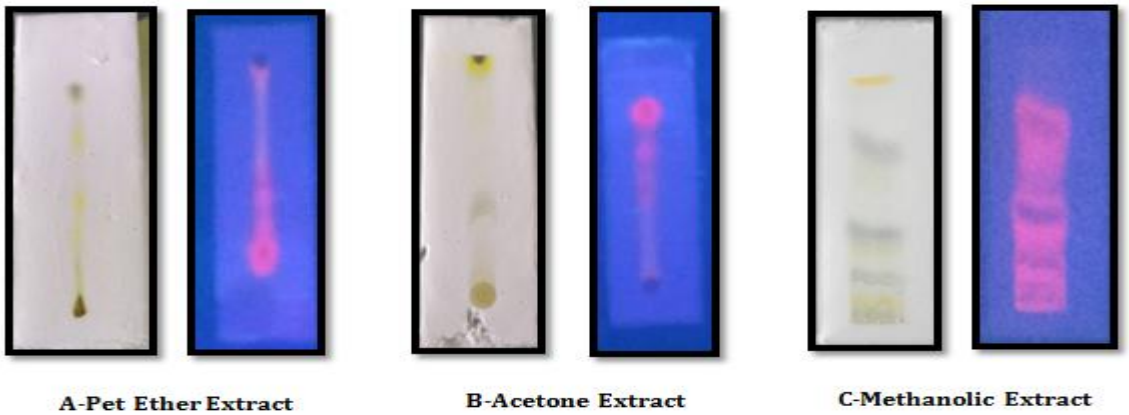

Fig. 2: TLC plates of A-pet ether,-Ethyl acetate and C-Methanolic extracts

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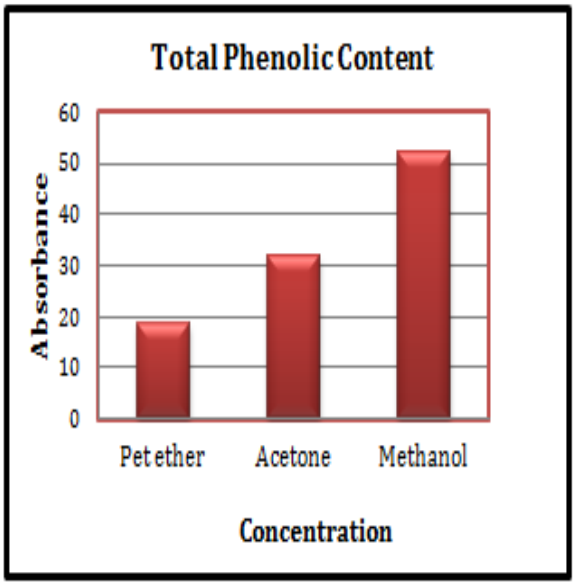
Table 2: Results of TLC profile of extracts:

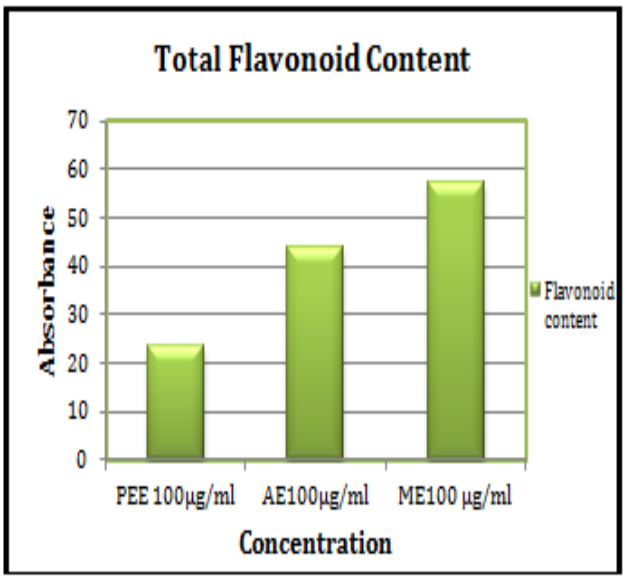
Sr. No.	Extracts	Solvent systems	Proportions	Spraying Reagent	R_f Values
1.	**Pet ether extract**	Benzene : Ethyl Acetate : Chloroform	(3.7 : 0.5 :0.8)	Sulphuric acid	0.07 0.25 0.12
2.	**Acetone extract**	Benzene: Chloroform: Ethyl Acetate	(5 : 3 : 2)	Sulphuric acid	0.07 0.24 0.10 0.32
3	**Methanol extract**	Toulene : Ethyl acetate: Ethanol (7:2:1)	(7:2:1)	Sulphuric acid	0.08 0.24 0.39

Table 3: Total phenolic content of *Terminalia catappa* leaves extracts

Sr. No.	Conc. µg/ml	Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg RE/g DW)
1	100	Petroleum ether	18.40 ± 0.26	23.22 ± 0.36
2	100	Acetone	31.93 ± 0.17	43.54 ± 0.20
3	100	Methanol	52.15 ± 0.44	57.41 ± 0.60



A-Phenolic Content



B-Flavonoid content

Chart 1: Total Phenolic and Flavonoid Content of T*erminalia catappa* leave extracts

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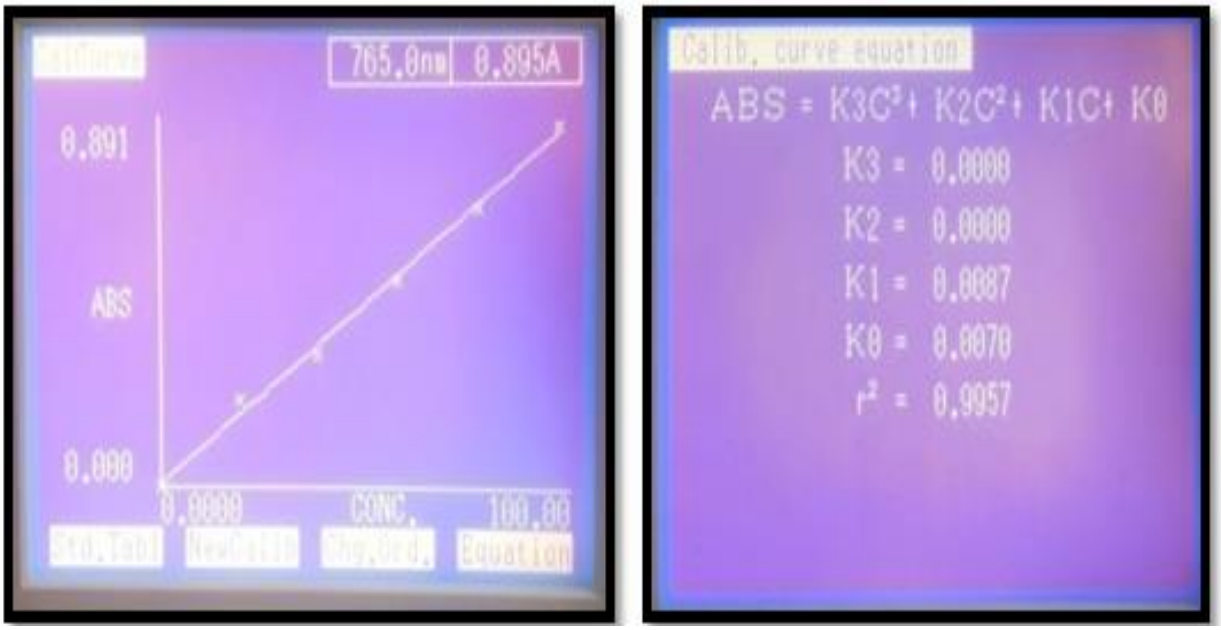
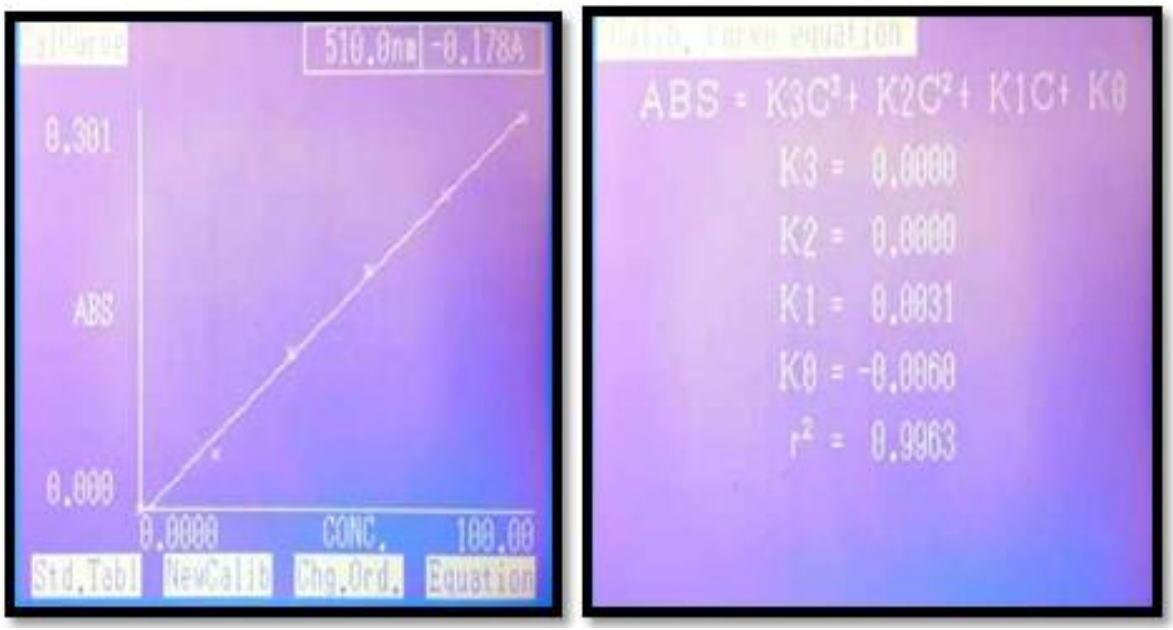


A B

Fig. 3: A-Calibration curve of Gallic acid ; B- Equation of Gallic acid



A B

Fig. 4: Clabration curve of Rutin; B- Equation of Rutin

3. Pharmacological evaluation of *Terminalia catappa* leaves extracts

***In-vitro* Anti-Oxidant Activity**

The antioxidant activity of *Terminalia catappa* was determined by *in-vitro* methods such as, DPPH free radical scavenging assay method. The assays were carried out in triplicate and average value was considered. The results were compared with Ascorbic acid as a reference standard.

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Table 4: DPPH (2, 2-Dipheny1, 1-Picrylhydrazyl) radical scavenging activity

Sr. No.	Conc. µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62 ± 0.23	41.06 ± 0.33	41.47 ± 0.19
2	50	73.51 ± 0.22	54.00 ± 0.26	51.54 ± 0.17
3	75	81.93 ± 0.21	76.18 ± 0.27	74.53 ± 0.25
4	100	87.88 ± 0.04	80.90± 0.27	79.05 ± 0.31
5	125	95.07 ± 0.25	91.17 ± 0.28	93.42 ± 0.31

Table 5: Comparative DPPH Scavenging assay method of *Terminalia catappa*

Sr. No.	Conc. µg/ml	Petroleum ether % inhibition	Acetone % inhibition	Methanol % inhibition	Ascorbic acid % inhibition
1	25	37.77 ± 0.15	59.74 ± 0.14	60.77 ± 0.20	62.62 ± 0.23
2	50	40.85 ± 0.20	69.39 ± 0.27	71.45 ± 0.15	73.51 ± 0.22
3	75	50.51 ± 0.23	78.02 ± 0.26	80.69 ± 0.16	81.93 ± 0.21
4	100	57.49 ± 0.15	83.98 ± 0.67	86.23 ± 0.19	87.88 ± 0.04
5	125	60.15 ± 0.16	85.67 ± 1.4	87.11 ± 0.21	93.07 ± 0.25

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity Concentration Vs % inhibition

From the above table and graph it reveals that all among all 3 extracts of *Terminalia catappa* Leaves, Methanol extract have comparable percent DPPH scavenging activity (87.11%) in comparison to standard ascorbic acid (93.07%). Methanolic extract shows better activity than the petroleum ether & Acetone extract.

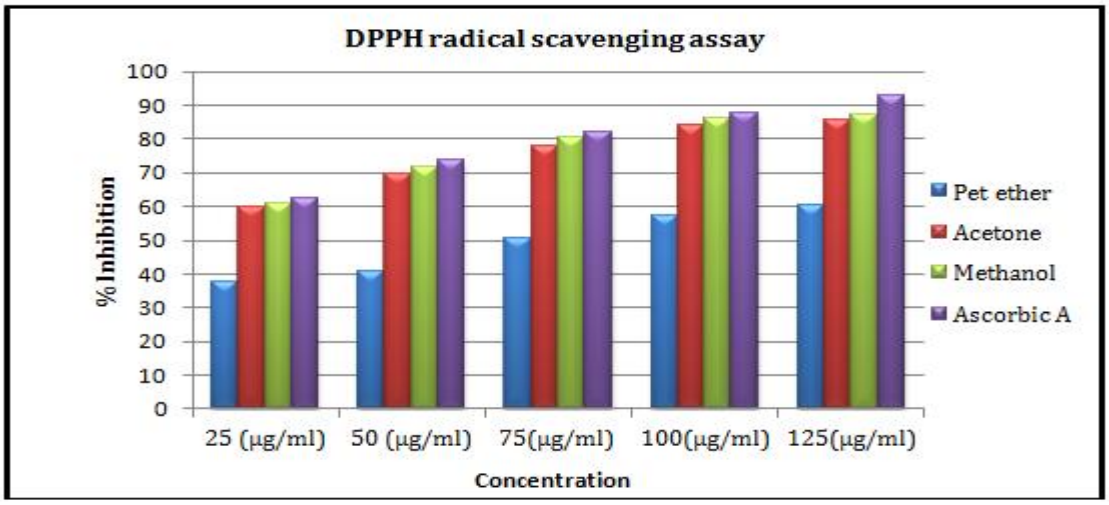

Chart 2: DPPH scavenging activity of *Terminalia catappa* leaves extracts

In DPPH scavenging activity, all the three extracts showed decrease in absorbance and increase in percentage inhibition as the concentration of extract was increased. All three extracts showed better activity at 125mg/ml & Methanolic extract *of Terminalia catappa* leaves has the maximum (87.11% inhibition) anti-oxidant activity as compared to pet ether and acetone extract.

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X. IN- VIVO ANTIANXIETY ACTIVITY

A) Elevated plus maze test

Table 6: *Terminalia catappa* Linn leaves extracts average readings from Elevated plus maze model

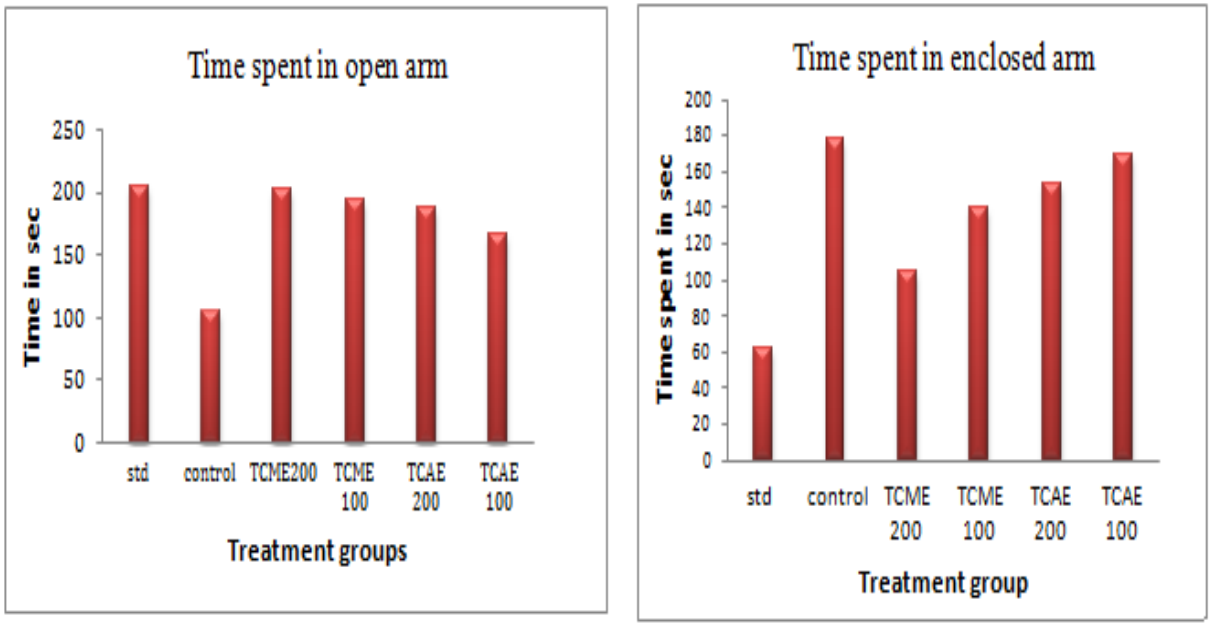
Treatment	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	No. of entries in open arm	No. of entries in enclosed arm	Time spent in central zone (sec)
DIAZEPAM (mg/kg)	205.1 ± 5.1	54.6 ±7.5	32.5 ± 4.5	10 ± 0.89	26 ± 3.6
Vehicle (DW)	104.5 ± 3.4	164.1 ± 5.1	4.8 ± 1.4	9 ± 3.1	17.1 ± 1.9
TC-ME (200mg/kg)	203 ± 3.1	113 ± 7.5	30.8 ± 4.9	8.5 ± 1.04	21.16 ± 2.7
TC-ME (100mg/kg)	195 ± 3.6	142.1 ± 6.0	25.5 ± 1.8	7.8 ±1.16	14.83 ± 1.6
TC-AE (200mg/kg)	187.3 ± 2.5	112.1 ± 6.0	18.5 ± 2	4.6 ± 1.03	20.8 ± 2.1
TC-AE (100mg/kg)	165.5± 8.4	146.3 ± 6.9	14 ± 2.5	5.8 ± 0.7	14.8 ± 1.4

Values are expressed as mean ± SEM (n = 6).

**p <0.001,*p<0.05 v/s Vehicle (One-way ANOVA followed by Tukey's test.)

The vehicle treated rat spent less time in open arm (104.8 ± 3.12 s) and more time in enclosed arm (164.1 ± 5.1 s) with 6.6 ± 1.6 entries in open arm and 9 ± 3.1entries in enclosed arm. The TC-AE (200mg/kg) &TC-ME (200mg/kg) show highly significant decrease in time spent in enclosed arm. Administration of TC-AE (100mg/kg), TC-ME (200mg/kg) & Diazepam (2mg/kg) show significant (p <0.01 & p<0.001) increase in number of entries in open arm.

The TC-AE & TC-ME (200mg/kg) and Diazepam (2mg/kg) show significant (p <0.001) increase in the occupancy in open arm indicating anxiolytic activity of TC-AE (200mg/kg) & TC-ME (200mg/kg) extract as compared to TC-AE (100mg/kg) & TC-ME (100mg/kg) extract.



A- Time spent in open arm **B- Time spent in enclosed arm**

Chart 3: Time spent in open arm and in enclosed arm (in sec) in elevated plus maze test

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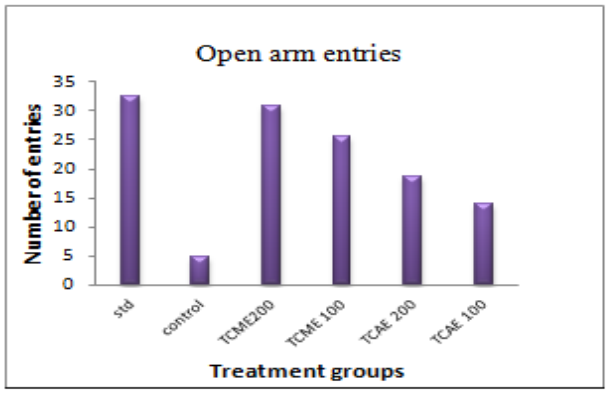
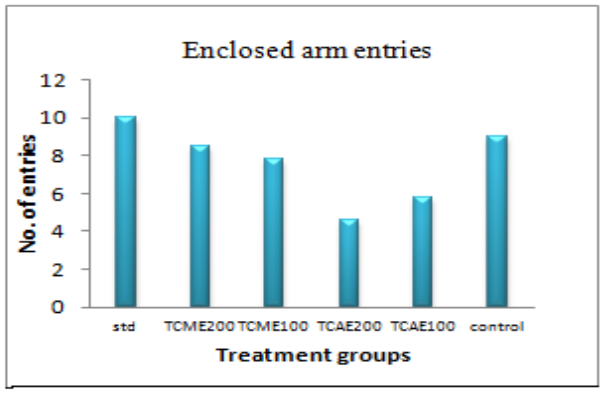




A- Total No. of entries in open arm **B-Total No. of entries in enclosed arm**

Chart 4: Total No. of entries in open and in enclosed arm in elevated plus maze test.

B) LIGHT & DARK TEST

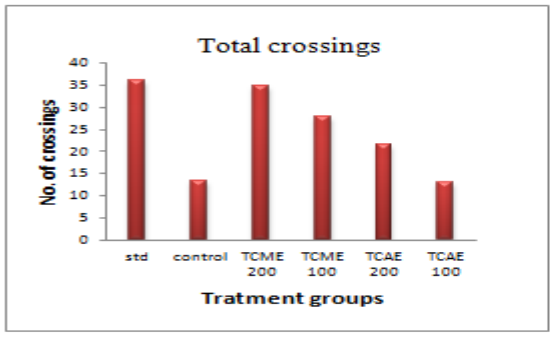
Table 7: Terminalia catappa Linn leaves extracts average readings from Light & Dark test

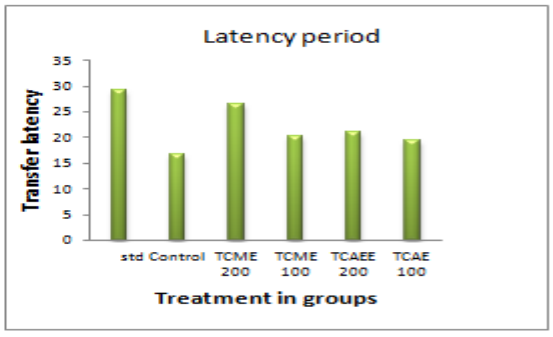
Treatment	No. of crossings	Time spent in dark zone (sec)	Time spent in light zone (sec)	Transfer latency
DIAZEPAM (2mg/kg)	36.16 ± 3.9	87.5 ± 5.6	203.1 ± 3.6	26.5 ± 1.5
Vehicle (DW)	13.3 ± 2.8	191.6 ± 4.17	104.8 ± 4.2	22.16 ± 2
TC-ME (200mg/kg)	34.6 ± 5.6	133.1 ± 5.5	161.5 ± 3.2	25 ± 1.4
TC-ME (100mg/kg)	28 ± 3	170.6 ± 3.3	124 ± 3	20 ± 1.4
TC-AE (200mg/kg)	21.5 ± 3.2	159.5 ± 4.8	143.16 ± 4	24.16 ± 2.3
TC-AE (100mg/kg)	12.8± 2.3	160.3 ± 5.5	132.5 ± 4	22.5 ± 1.8

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

The animal treated with TC-ME (200mg/kg) & Diazepam (2mg/kg) show highly significant (p <0.001) and TC-AE(200mg/kg) show significant increase in time spent in light zone & decrease in time spent in dark zone. Administration of TC-ME (200mg/kg) & TC-AE (200mg/kg) show significant decrease in time spent in dark zone as compared to the vehicle group.

Animal treated with TC-ME (200mg/kg) &TC-AE (200mg/kg) show increase in no. of crossing & transfer latency as compared to vehicle group in light & dark test indicating the anxiolytic activity of TC-ME (200mg/kg) &TC-AE (200mg/kg) extract.





A- Total crossings **B-Latency period in Light and dark test.**

Chart 5: Total crossings and Latency period in Light and dark test.

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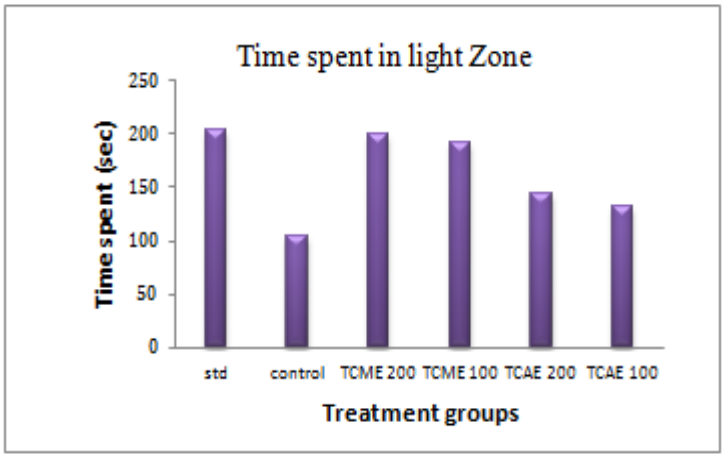

Chart 6: Time spent in light zone

IX. DISCUSSION

In the last two decades of the century, the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. Different parts of this plant have been reported to possess anti-inflammatory, antidiabetic, anti-oxidant, hypolipidaemic, antiobesity and antimicrobial activity [9]. The leaves are cooling, emollient, anti-pyretic, hypoglycemic, diuretic, Laxative, digestible, anthelminthic, urinary concretions, sore throats, pain in the joints, flatulence throat [10].

Traditionally it has been reported that, *Terminalia catappa* leaves may exhibit anti-inflammatory potential so it was selected for evaluation of antianxiety studies.

The Preliminary phytochemical evaluation of Acetone & methanolic extracts were carried out for the determination of presence of phytoconstituents along with TLC fingerprinting. Both extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids, and saponins [11].

Antioxidant property of *Terminalia catappa* leaves extracts was carried out by using DPPH radical scavenging assay technique. In this method percentage inhibition of test sample was calculated and compared with percentage inhibition of standard (ascorbic acid). By this method the percentage inhibition shown by the acetone & methanolic extracts were 85.67% and 87.11% respectively.This provides evidence that methanolic extract of *Terminalia catappa* leaves has potent antioxidant activity and it can be used as an antioxidant agent.

The Antianxiety activity of acetone and methanolic extracts of *Terminalia catappa* leaves was evaluated in rats by daily exposing them to the Elevated plus maze and light and dark model. Results obtained were compared with standard drug Diazepam (2mg/kg).

In Elevated plus maze model rats were allowed to spent time in it for 5min and time spent in open arm, enclosed arm and central zone with no. of entries in enclosed arm and open arm were considered as evaluation parameters. In Light and Dark model, rats were allowed to move from two chambered system i.e. from light zone to dark zone and behavioural chages were observed with transfer latency and time spent evaluation parameters. The results showed that the highest dose (200mg/kg) of both the extracts, showed highly significant antianxiety activity when given orally in daily single dose. The findings suggest that the effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through behavioural changes of the animals. At the end of the study it was observed that acetone and Methnolic extracts of plant shows significant difference as compared to vehicle or control treated group and shows behavioural effects same as produced by standard drug Diazepam. From the results it was revealed that both extract i.e. acetone (100mg/kg), (200mg/kg) and methanolic (100mg/kg), (200mg/kg) showed effective Antianxiety activity.

X. SUMMARY AND CONCLUSION

Terminalia catappa leaves contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like cancer, inflammation, infectious, cardiopathy, diabetes, hepatotoxicity and many microbial attacks where its memory enhancing potential is claimed to be useful. No methodical reports on antianxiety activity of *Terminalia catappa* leaves were available. The present study aimed at evaluating the *In-vivo* Antianxiety of *Terminalia catappa* leaves extract in rats. Acetone and methanolic extracts were

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prepared by the hot extraction process, i.e. by using Soxhlet apparatus. Preliminary phytochemical evaluation of Acetone and methanolic extract was carried out for the determination of presence of phytoconstituents.

Antioxidant property of *Terminalia catappa* leaves was carried out by using DPPH radical scavenging assay technique. In DPPH assay all the extracts showed promising antioxidant activity, however Methanolic extract of *Terminalia catappa* leaves revealed significant antioxidant activity. The result of acute oral toxicity studies of plant extracts as per standard references revealed that in single dose the plant extracts had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in rat. Accordingly safe experimental dose was calculated as ≤ 200mg/kg & was used accordingly for further screening of extracts. *In-Vivo* study has showed that acetone and methanolic extracts of *Terminalia catappa* does possess significant Antianxiety activity with 100 mg/kg and 200 mg/kg, but high doses of the acetone extract 200 mg/kg being more superior and showed significant to highly significant percentage inhibition (from $P < 0.05$ to $P < 0.001$) when compared with standard Diazepam. The finding of the present study reveals that *Terminalia catappa* leaves has potent antianxiety activity.

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