



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

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ABSTRACT

The present study reports physicochemical characterization, antioxidant and Antiamnetic 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 water 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 primary and secondary metabolites 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 leaf extracts. The *In-Vivo* antiamnetic activity of *Terminalia catappa* leaf was evaluated by radial arm maze model in rats using Piracetam as a standard. Scopalamine (1mg/kg) used as inducing agent. Both the extracts at 200mg/kg concn showed significant to highly significant number of entries & time spent in P zone (from $P < 0.05$ to $P < 0.001$).

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

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I. INTRODUCTION:

A French psychologist Theodule-Armand Ribot was among the first scientists to study amnesia. Because of this, medical experts started to call the gradients of memory loss as Ribot gradients. Amnesia is when a person can no longer memorize or recall information that is stored in memory. It is very rare, despite being a popular theme for movies and books. Being a little forgetful is completely different to having amnesia. Amnesia refers to a large-scale loss of memories that should not have been forgotten. These may include important milestones in life, memorable events, key people in our lives and vital facts we have been told or taught.

Areas include the medial temporal lobe structures comprising the hippocampus, the amygdala, the entorhinal cortex and the perirhinal cortex. A second brain region associated with amnesia is the midline diencephalic region, which includes the mamillary

bodies of the hypothalamus and the dorsomedial nucleus of the thalamus. Causes of damage to these diencephalic structures include stroke, brain tumours in this region [1].

***Terminalia catappa* Linn.** Belongs to the family (Combretaceae) and is popularly known as 'deshi badam'. It is a well known herb in Ayurvedic system of medicine. Juice of young leaves are employed in preparation of ointment for leprosy, scabies and also used internally for colic and headache. Aqueous and ethanolic extracts of leaves were reported for their hepatoprotective activity. The tree grows to a height of 35 m with an upright, symmetrical crown and horizontal branches. Its branches are characteristically arranged in tiers. The leaves are large, 15-25 cm long and 10-14 cm broad, ovoid, glossy dark green, and leathery. The trees are monoecious, with distinct male and female flowers on the same tree. Both are 1 cm in diameter, white to greenish and inconspicuous with no petals. The leaves

of *T. catappa* contain 1-degalloyl-eugenin, 2,3-(4,4',5,5',6,6'-hexahydroxy-diphenyl)-glucose, chebulagic acid, gentisic acid, corilagin, geraniin, granatin B, kaempferol, punicalagin, punicalin, quercetin, tercatatin, tergalagin, terflavin A, and terflavin B. [2].

The main of this article is phytochemical and pharmacological evaluation of *terminalia catappa* linn. leaves extracts for antiamnetic 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 Professor & 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.

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.

Preparation of Extract [3]:

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 leaf was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 150gm. of the powdered and 1000 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 [3].

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.

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.

4. 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.

5. 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.

6. 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 magenta color is observed.

7. 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.

8. 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 FINGERPRINTS [3]:**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°C for one hour. Activation of TLC plates is nothing but removing water/moisture and other substances from the surface of any adsorbent, by heating at temperature around 110°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, development distance, temperature, amount of substance added, and the presence of impurities.

Distance from origin to the point of maximum intensity

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

R_f = Retention factor

2. Total Phenolic Content [4, 5].

Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1ml) 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 [6, 7]:

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.

1. 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 vortexed and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A

Evaluation:

The evaluation was carried out on 8th day 24 hr after the respective drug treatment where food pellets were placed in variable arm for evaluation of working memory.

The Number of errors (Entries to non-baited arms) counted during the session.

Animal Grouping and drug administration:

Wistar rats of either sex weighing 150-200gm., obtained from animal house of college. The Animals were randomly divided into seven groups of six animals in each as follows:-

Table 1: Grouping of animals

Sr no.	Groups	Animals (<i>Wistar rats</i>)
1	Control group (Vehicle-treated)	Six rats
2	Standard (Piracetam 200 mg/kg ip)	Six rats
3	Scopolamine (1mg/kg ip)	Six rats

4	Acetone + Scopolamine (100mg/kg orally)	Six rats
5	Acetone + Scopolamine (200mg/kg orally)	Six rats
6	Methanolic+Scopolamine. (100mg/kg orally)	Six rats
7	Methanolic+Scopolamine. (100mg/kg orally)	Six rats

VIII. STATISTICAL ANALYSIS

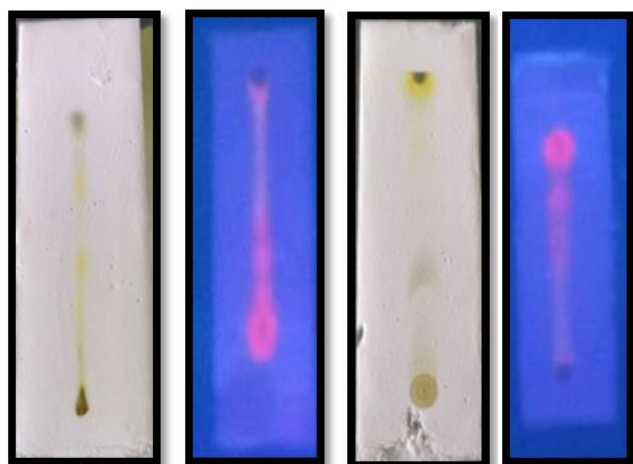
The data were expressed as mean + standard of mean (SEM). Statistical analysis were performed by one way analysis of variance (ANOVA).

XI. RESULTS:**1. Phytochemical tests of *Terminalia catappa* leaves extract:****Table 2: 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	+	+	+
	Steroids			
8	Salvoski test	-	+	+
	Libberman test	-	-	+
9	Amino acid			
	Ninhydrin test	+	+	+
	Tyrosin test	+	-	-
	Tryptophan test	-	+	+

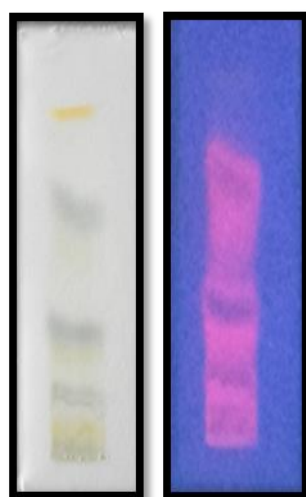
Table 4: Total phenolic content of *Terminalia catappa* leaves extracts

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-Pet Ether Extract

B-Acetone Extract



C-Methanolic Extract

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

Table 3: Results of TLC profile of extracts:

Sr. No.	Extracts	Solvents	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

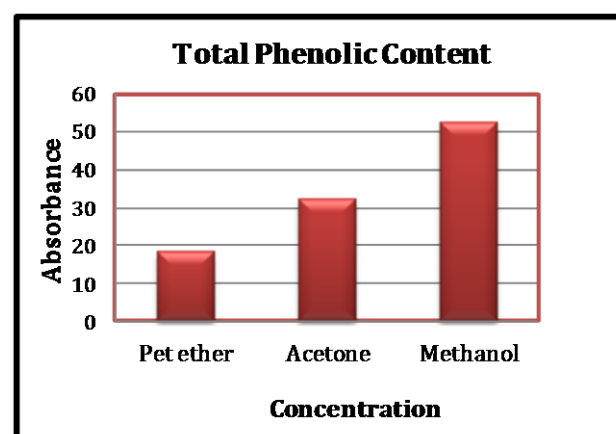
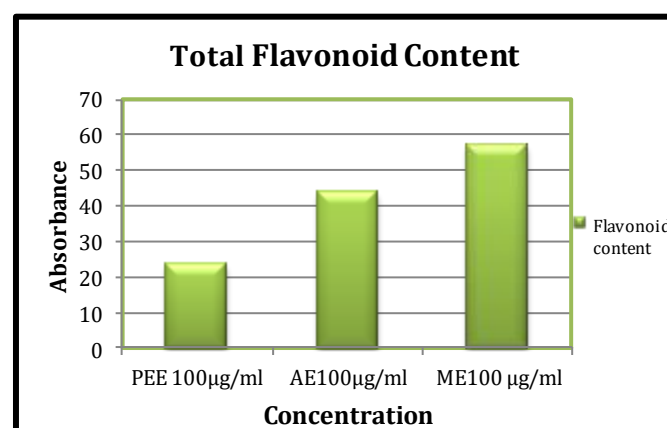
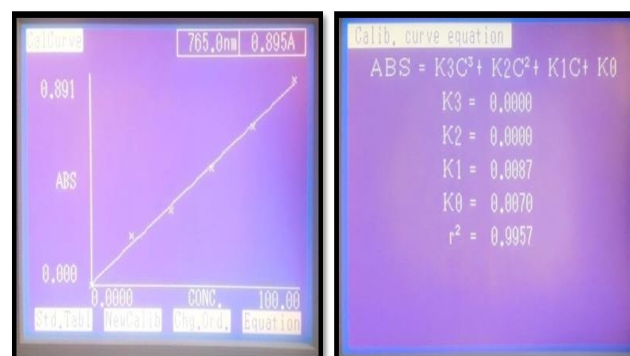
Chart 1: Total Phenolic content of *Terminalia catappa* leaf extractsChart 2: Total Flavonoid Content of *Terminalia catappa* leaf extracts

Fig. 3: Calibration Curve of Gallic acid

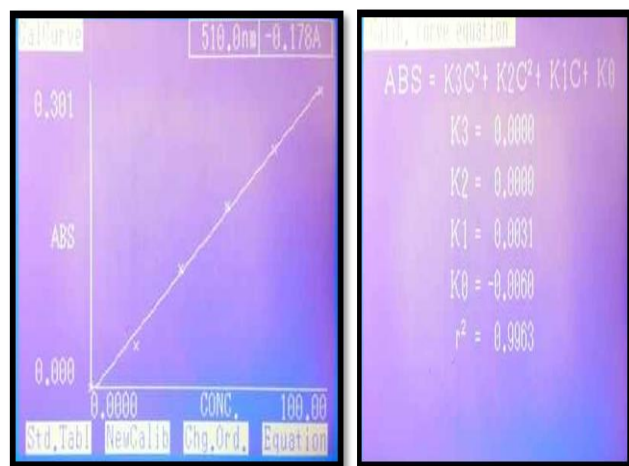


Fig. 4: Calibration Curve of Rutin

2. 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.

Table 5: DPPH (2, 2-Diphenyl, 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 6: 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-diphenyl 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.

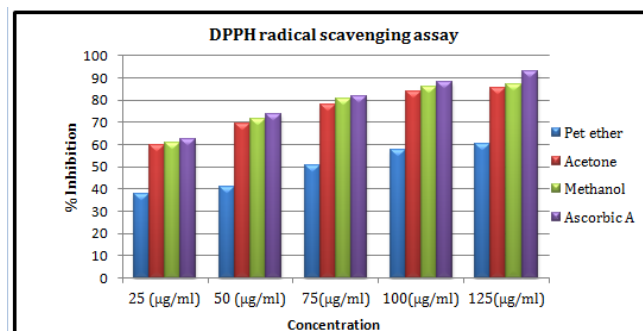


Chart 3: DPPH scavenging activity of *Terminalia catappa* leaves extracts

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.

3. In- Vivo Antiamnetic activity

Number of entries in C and P zone of rats for Antiamnetic activity of *Terminalia catappa* Acetone (TCAE) and Methanolic (TCME) leaves extracts.

Table 7: Number of Entries in C zone

Sr. no.	Group name	Number of Entries in C zone	
		Day 1	Day 8
1	Std	12.16±0.47	10.5±0.42**
2	Ctrl	21.5±0.92	18.55 ±0.42
3	Scopolamine	11.3±1.4	12.66 ±1.211
4	TCAE-100+Sco.	19.16±0.47	16.5±0.42*
5	TCAE-200+Sco.	18.16±0.30	15.83±0.47*
6	TCME-100+Sco.	16.16±0.47	14.83±0.30* *
7	TCME-200+Sco.	14.16±0.47	12.16±0.60* *#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

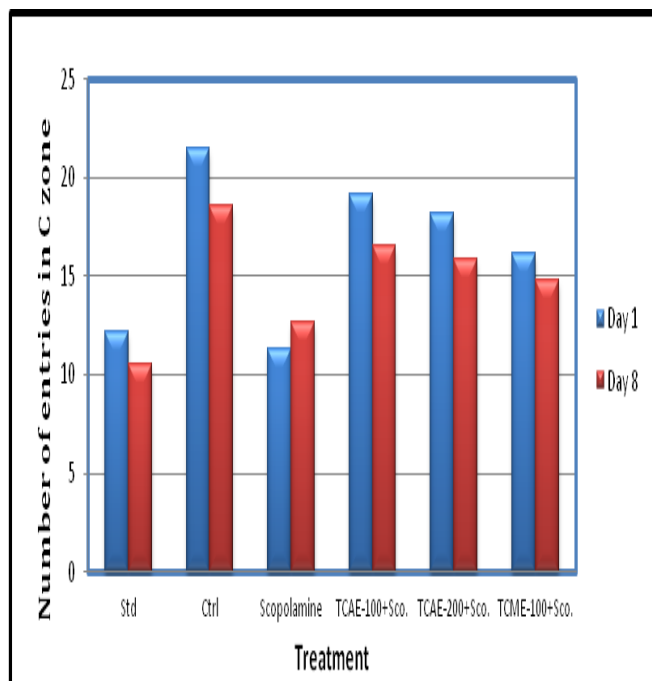


Chart 4: Number of entries in C zone of rats during experimental period

The number of entries in C zone on day 8 when compared with day 1 it was found that TCAE-100, TCAE-200 & TCME-100, TCME-200 shows no significant difference as compared to control but standard shows highly significant difference on day 8. All test doses shows no significant difference when compared with Standard.

Table 8: Number of Entries in P zone

Sr. no.	Group name	Number of Entries in P zone	
		Day 1	Day 8
1	Std	8.66±0.21	10.5±0.42**
2	Ctrl	3.5±0.22	3.83±0.16
3	Scopolamine	4.6 ±0.16	8.6±0.23
4	TCAE-100+Sco.	4.66±0.21	5.5±0.22*
5	TCAE-200+Sco.	5.16±0.30	6.33±0.22*
6	TCME-100+Sco.	7.16±0.30	7.33±0.42**
7	TCME-200+Sco.	8.16±0.30	9.16±0.47**#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

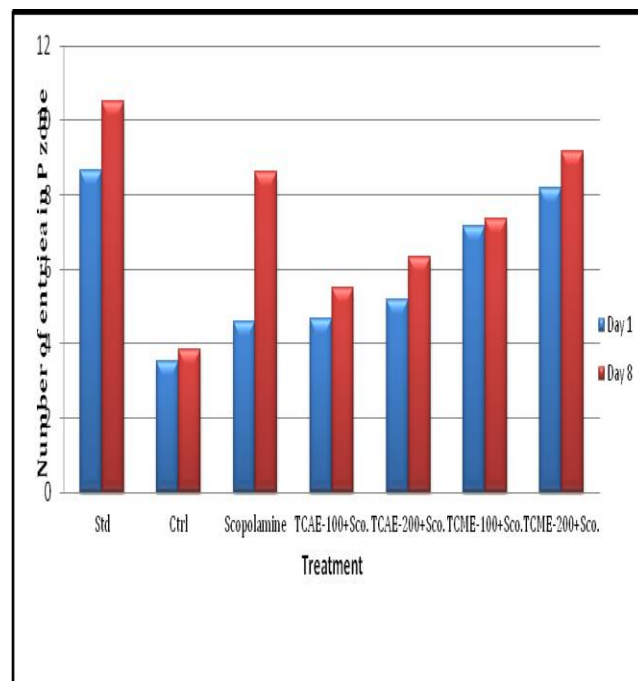


Chart 5: Number of entries in P zone of rats during experimental period

The number of entries in P zone on day 8 when compared with day 1 it was found TCAE-100, TCAE-200 & TCME-100, TCME-200 shows highly significant difference as compared to control (P<0.001). All test doses shows no significant difference when compared with Standard.

Table 9: Time spent in C and P zone of rats for Antiamnetic activity of *Tareminalia Catappa* (Acetone and Methanolic) leaf extracts

Sr. No.	Group name	Time spent in C zone	
		Day 1	Day 8
1	Ctrl	32.33±0.55	30.33±0.42**
2	Std	65.33±1.25	66.16±1.51
3	Scopolamine	26.5±1.61	32.66±1.3
3	TCAE-100+ Sco.	46.16±0.47	53.16±0.54**
4	TCAE-200+ Sco.	42.16±1.16	43.16±0.47**
5	TCME-100+ Sco.	40.5±0.76	35.16±0.47**
6	TCME-200+ Sco.	36±0.57	33.16±0.47**#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

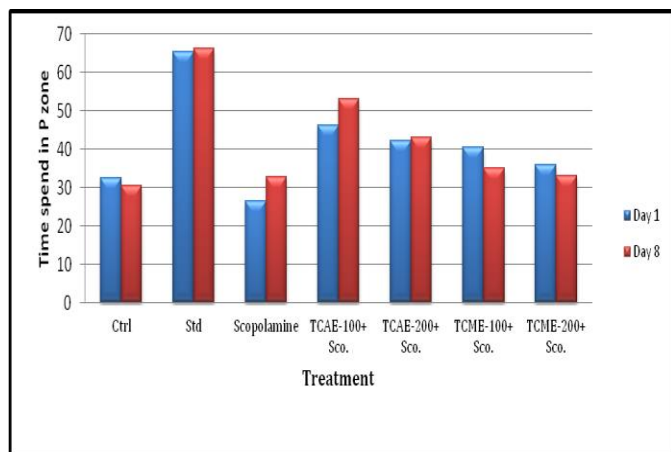


Chart 6: Time spent in C zone of rats during experimental period

The time spent in P zone on day 8 when compared with day 1 it was found that TCAE-100, TCAE-200 & TCME-100, TCME-200 shows highly significant difference as compared to control ($P < 0.001$). All test doses shows no significant difference when compared with Standard.

Table 10: Time spent in P zone

Sr. No	Group name	Time spent in P zone	
		Day 1	Day 8
1	Ctrl	145.83±0.87	170.33±1.72**
2	Std	112.33±1.58	137.33±0.66
3	Scopolamine	109.34±1.60	117.34±0.58
3	TCAE-100+ Sco.	134.16±0.65	160.16±0.65**
4	TCAE-200+ Sco.	138.16±0.47	163.16±0.65**
5	TCME-100+ Sco.	139.16±0.60	165.5±0.99**
6	TCME-200+ Sco.	143.66±0.71	167±0.57**#

The values are represented as mean \pm S.E.M ($n=6$) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * $P < 0.05$ -Significant difference when compared to control, ** $P < 0.001$ - Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ -Significant difference when compared to Standard but more activity.

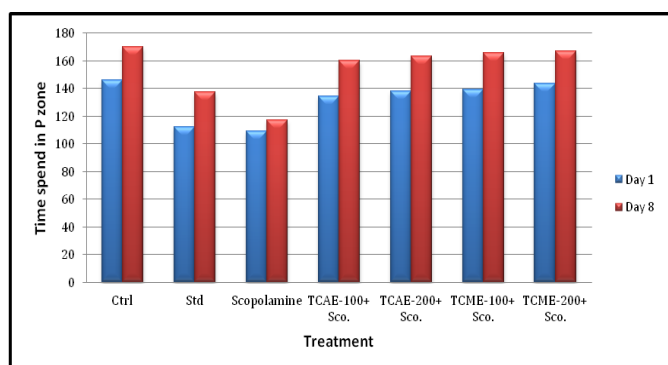


Chart 7: Time spent in C zone of rats during experimental period

The time spent in C zone on day 8 when compared with day 1 it was found that the TCAE-100 and TCME-100 shows significant difference when compared to control ($P < 0.05$) but TCAE-200 & TCME-200 shows highly significant difference ($P < 0.001$). All test doses shows significant difference when compared with Standard.

X. 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, anthelmintic, urinary concretions, sore throats, pain in the joints, flatulence throat. Traditionally it has been reported that, *Terminalia catappa* leaves may exhibit anti-inflammatory potential so it was selected for evaluation of anti-inflammatory studies [10].

Preliminary phytochemical evaluation of acetone & Methanolic extracts was 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. The spots at R_f values (pet ether) 0.07, 0.25, 0.12 and (Acetone extract) 0.07, 0.24, 0.10, 0.32 (Methanolic extract) 0.08, 0.24, 0.39 represents the presence of kaempferol, punicalagin, punicalin, quercetin, tercatatin, in the extracts.

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). 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%). This provides evidence that Acetone and Methanol extract of *Terminalia catappa* leaves has potent antioxidant activity and it can be used as an antioxidant agent.

In-vivo memory enhancing activity of *Terminalia catappa* leaves extracts was evaluated by using the Radial arm maze and by using Wistar rats as an animal model.

The anti-amnesic activity of acetone and methanolic extracts of *Terminalia catappa* leaves was evaluated in rats by daily exposing them to the radial arm maze with the food pellet in a fix arm of maze. Food pellets were placed in a variable arm for evaluation of working memory. It is characterized by increase in latency to find the food and time spent in selected arm. The results were drawn by evaluating time spent & number of entries in P zone.

The results showed that the highest dose (200mg/kg) of both the extracts, showed highly significant memory enhancing activity when given orally in daily single dose. The findings suggest effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through ability of the animals to cause a significant decrease in number of errors and increase in latency to find the food and time spent in selected zone as well.

At the end of the study it was observed that group no. 7 i.e. Methanol extract treated group at dose of 200mg/kg showed maximum number of entries at P zone (**9.16±0.47**#**). The same group showed maximum time spent at P zone (**167±0.57**#**) as well. Group no.7 (Methanol at dose of 200mg/kg) and group no. 6 (Methanol at dose of 100mg/kg) represent **9.16±0.47**#**, **7.33±0.42**#** for number of entries in P zone. Time spend of these groups at P zone were found to be **117.34±0.58**, **160.16±0.65****, **163.16±0.65****, **165.5±0.99****, **167±0.57**#** respectively. All these values were compared with standard drug i.e. Piracetam at dose of 200mg/kg.

From the results it was revealed that both extract i.e. acetone and methanolic showed effective antiamnetic activity. Although Methanolic extract at 200 mg/kg showed more superior and significant to highly significant (from $P < 0.05$ to $P < 0.001$) antiamnetic activity by using radial arm maze in rats.

XI. 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 Depression, Anti-inflammatory, Anti-bacterial, Anti-oxidant, Anti-tumor, Anti-fungal, Antiviral and many microbial attacks where its antiamnetic potential is claimed to be useful. The extracts of *Terminalia catappa* leaves tested for antiamnetic activity by researchers. No methodical reports on antiamnetic activity of *Terminalia catappa* leaves were available.

The present study aimed at evaluating the *In-vivo* antiamnetic activity of *Terminalia catappa* leaves extract in rats. Aqueous and methanolic extracts were 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 respectively. In DPPH assay all the extracts showed promising antioxidant activity, however acetone 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 ≤ 200 mg/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 antiamnetic activity with 100 mg/kg and 200 mg/kg, but high doses of the Methanolic 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 Piracetam.

The finding of the present study reveals that further study is requiring evaluating the mode of action of antiamnetic effect of *Terminalia catappa* leaves extracts.

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