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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *TERMINALIA CATAPPA* LEAVES EXTRACTS FOR ANTIDEPRESSANT ACTIVITY

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ABSTRACT

The present study reports physicochemical characterization, antidepressant 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. In-vivo Antidepressant effect of *Terminalia Catappa* Linn. Leaves was determined by Despair Swim test method using Imipramine (15mg/kg orally) as standard were two extracts as 200mg/kg Concentration showed significant to highly significant result of decrease in time of immobility on 8th and 15th day of experiment.

Keywords: *Terminalia Catappa Linn.,* Acetone and methanol extract, Phytochemical screening, Antioxidant effect, Antidepressant activity, Despair Swim test.

I. INTRODUCTION:

As estimated by WHO, depression shall become the second largest illness in terms of morbidity by another decade in the world, already one out of every five women, and twelve men have depression. Not just adults, but two percent of school children, and five percent of teenagers also suffer from depression, and these mostly go unidentified. Depression has been the commonest reason why people come to a psychiatrist, although the common man's perception is that all psychological problems are depression [1-2]. What one sees in most patients is the myth related to depression. People still believe that it is because of some weakness in personality, or that one can cure it by oneself, or that medication would go lifelong and are mere sedatives. All these are myths, and mostly created by faith healers, or unqualified counselors, and non-medical experts for their own vested interest, and largely by an unaware of society. An increased awareness, and approach to psychiatrists, has been the main reason for the increase in number of patients and not necessarily an increase in prevalence. With newer medication, and better facilities, treating depression has become easier, and most people respond very well to treatment, and return to optimum functioning very soon [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. Acetone and methanolic extracts of leaves were reported for their hepatoprotective activity. Reddish brown leaves contain flavonoid apigenin 6-c-(2 - galloyl)- L-Dglycoside, apigenin 8-c-(2 - galloyl)- L-Dglycoside, isovitexin, vitexin rutin and tannin ; gallic acid, ellagic acid, punicalin which are reported for good antioxidant property. Antidiabetic potential of T. catappa

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fruits has been investigated for its effect on fasting sugar level and serum parameters [2].

More and more pharmacological studies have reported that the extract of *Termialia catappa* leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti- inflammatory, antidiabetic effects and hepatoprotective activities, but the effective components and related mechanisms remain unknown. Recently, data from our laboratory revealed that the chloroform fraction of the ethanol extract of *T. catappa* leaves has the strongest anti-inflammatory activity among all fractions from ethanol extract of T. catappa leaves. The tree grows to a height of 35 m with an upright, symmetrical crown and horizontal branches. Its branches are characteristically arranged in tears. 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 fruit is a drupe 5-7 cm long and 3-5.5 cm broad, green at first, then yellow, and finally red when ripe, containing a single seed. The seed within the fruit is edible when fully ripe [2].

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 [3]:

Three extracts of *Tarminalia 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 [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.

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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 diluted 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 magnetic 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 FINGERPRINT [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^{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, 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 [4,5].**
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 [4,5].**
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.

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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

Methodology:

The rats were fasted overnight and maintained 85% of its total diet weight. Animals were divided randomly into six treatment groups; each group consisting of six rats, each treatment group received orally the extracts of *T. Catappa* leaves in a dose of 100 mg/kg and 200mg/kg of both the extract as per the group. Extracts was given to rats, once daily for period of 8 days and daily evaluation was done. The end point will be taken as per the number of entries in P zone and time spent in P zone. The mean of number of entries and time spent in P zone for each group is calculated.

VI. ANTIDEPRESSANT MODEL (DESPAIR SWIM TEST MODEL) [8]:

Purpose & rationale:

Behavioral despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in restricted space from which they cannot escape are induced to a characteristics behavior of immobility.

Animal Grouping and drug administration:

Group I	Control (1% DMSO) orally
Group II	Standard (Imipramine 10 mg/kg) orally
Group III	Terminalia Catappa Linn. Acetone extract (TCAE) 100mg/kg orally
Group IV	Terminalia Catappa Linn. Acetone extract (TCAE) 200mg/kg orally
Group V	Terminalia Catappa Linn. Methanol extract (TCAE) 100mg/kg orally
Group VI	Terminalia Catappa Linn. Methanol extract (TCAE) 200mg/kg orally

Procedure:

Wistar rats of either sex weighing 160–180 g will be used. They will be brought to the laboratory at least one day before the experiment and will be housed separately in cages with free access to food and water. Rats will be individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C). Rats placed in the cylinders for the first time will be initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The total duration of immobility was recorded. After 6 min in the water the rats are removed and dried with cloth before being returned to their home cage. The water is changed after each test because urine and the other chemicals released by the first rat were affecting the swimming pattern of the next rat.

An animal is judged to be immobile whenever it remains floating passively in the water in slightly hunched but upright position, its nose just above the surface. Plant extract or standard drug imipramine (15mg/kg) is administered one hour prior to testing. The similar procedure was conducted 1st, 8th, and 15th days of experiment.

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Evaluation

Evaluation was done on the basis of duration of immobility time in sec in total 6 min test on respective of experiment i.e. on day 1st, 8th and 15th day.

VIII. STATISTICAL ANALYSIS

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

XI. 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 soln test:	+	+	+
	Dil.Nitric acid test	+	-	+
6	**Flavonoids**			
	Alkaline test	+	-	+
	Shinoda test	+	+	+
	Steroids			
8	Salwoski test	-	+	+
	Libberman test	-	-	+
9	**Amino acid**			
	Ninhydrin test	+	+	+
	Tyrosin test	+	-	-
	Tryptophan test	-	+	+

2. TLC Fingerprinting:

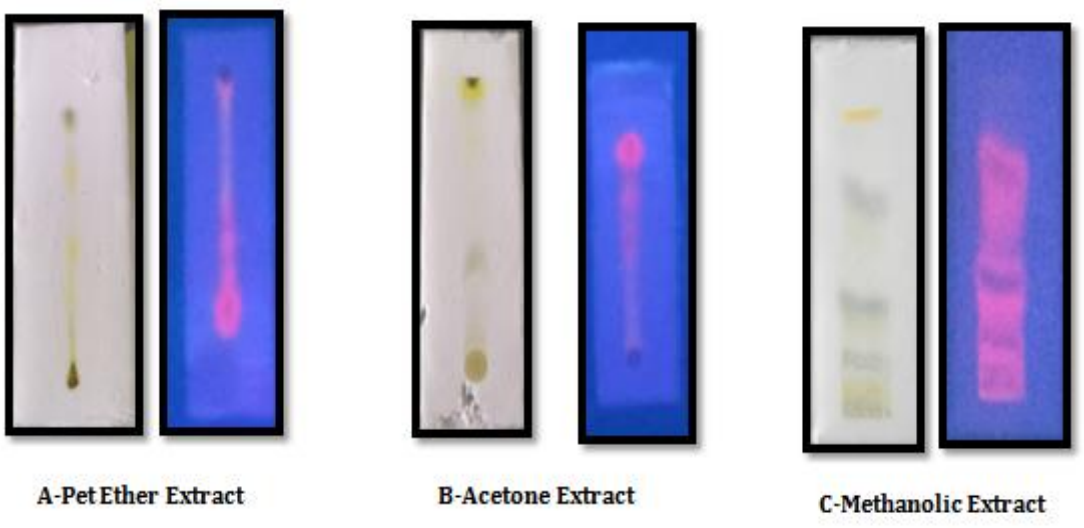

Fig. 1: TLC plates of pet ether, Acetone and Methanolic extracts

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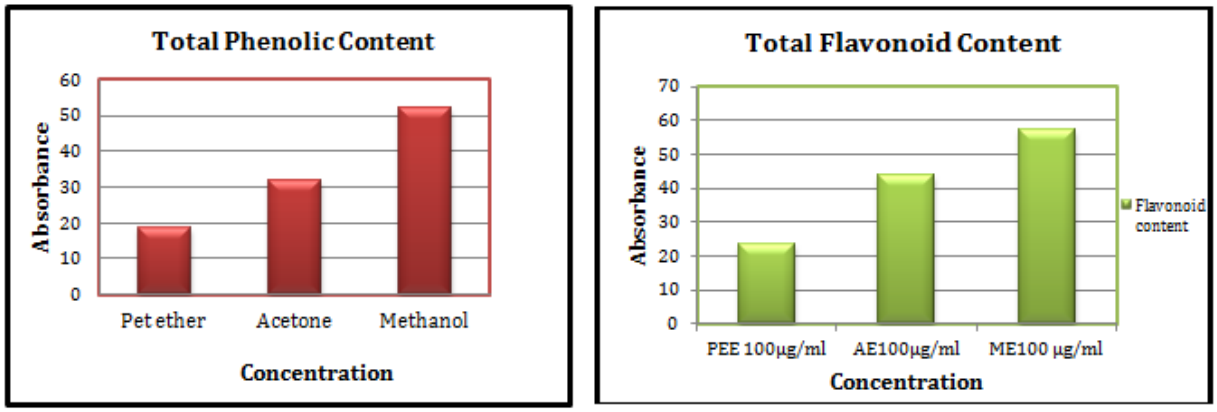
Table 2: Results of TLC profile of extracts

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

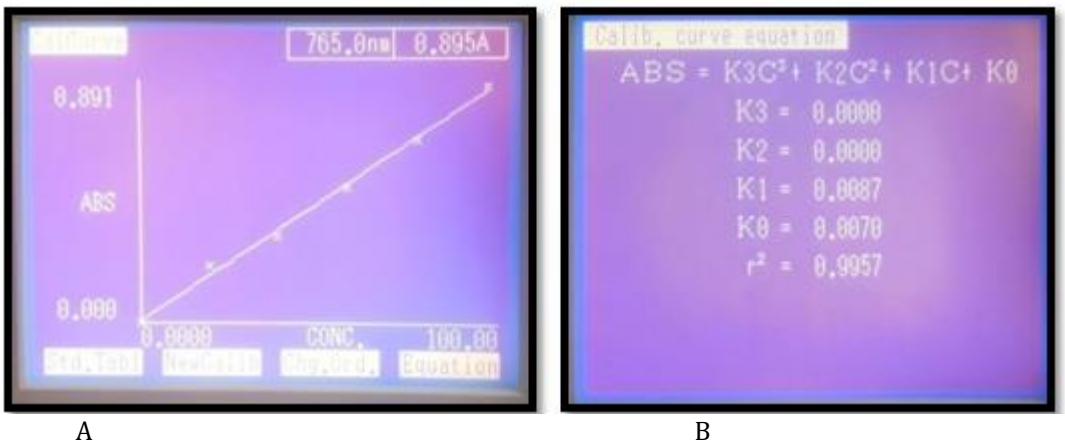
Table 3: Total phenolic content of *Terminalia catappa* leaves extracts

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 (A) and Flavonoid (B) content of Terminalia *catappa* leave extracts



A B

Fig. 2: A-Calibration curve of Gallic acid; B-Calibration Equation of Gallic acid

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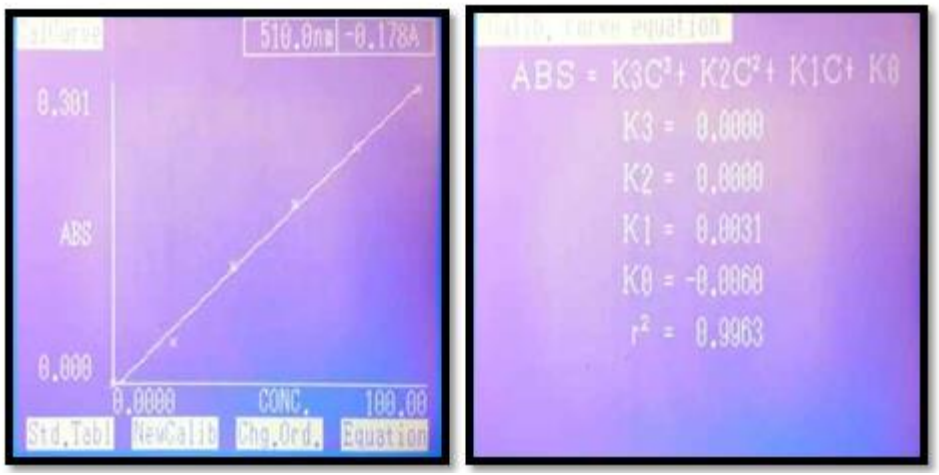

A B

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

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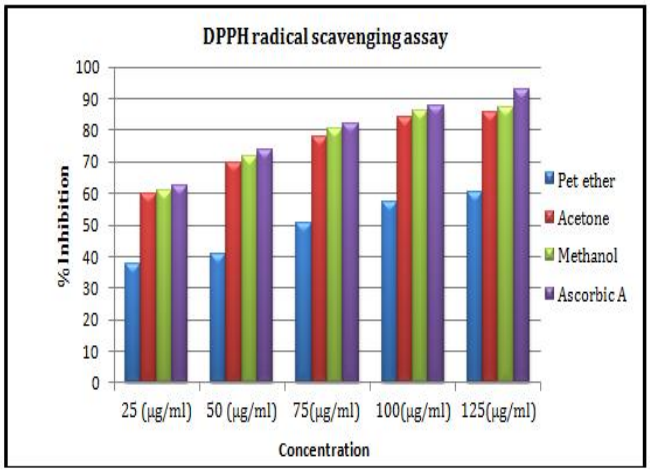

Chart 2: DPPH scavenging activity of *Terminalia catappa* leaves extracts

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.

Pharmacological Evaluation of *Terminalia Catappa Linn.leaves* extracts.

Table 6: Effect of Day 1 TCAE and TCME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 1
Control (DMSO 2%)	86.66 ± 1.14
Imipramine 10 mg/kg	44.83 ± 1.35 ***
TCAE 100 mg/kg	62.5 ± 1.60 ***
TCAE 200 mg/kg	57.33 ± 1.60 ***
TCME 100 mg/kg	52.33 ± 1.60 ***
TCME 200 mg/kg	45.16 ± 0.94 ***#

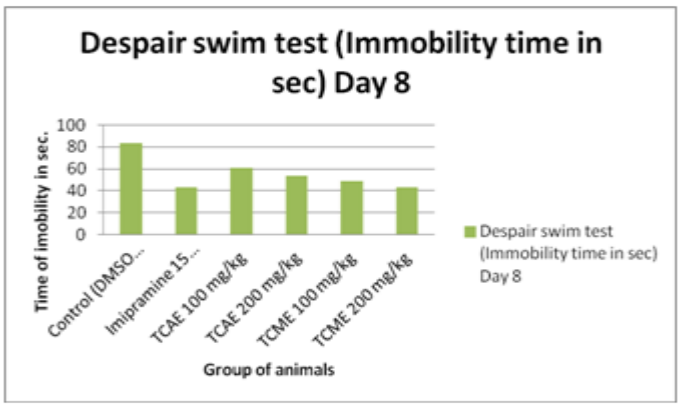

Chart 3: Despair swim day 8 tests of *Terminalia Catappa Linn.leaves* extracts.

Table 7: Effect of Day 8 TCAE and TCME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 8
Control (DMSO 2%)	83.83 ± 1.49
Imipramine 15 mg/kg	42.83 ± 1.53***
TCAE 100 mg/kg	60.83 ± 0.94***
TCAE 200 mg/kg	53.66 ± 1.85 ***
TCME 100 mg/kg	48.5 ± 1.47 ***#
TCME 200 mg/kg	43.5 ± 1.60 ***#

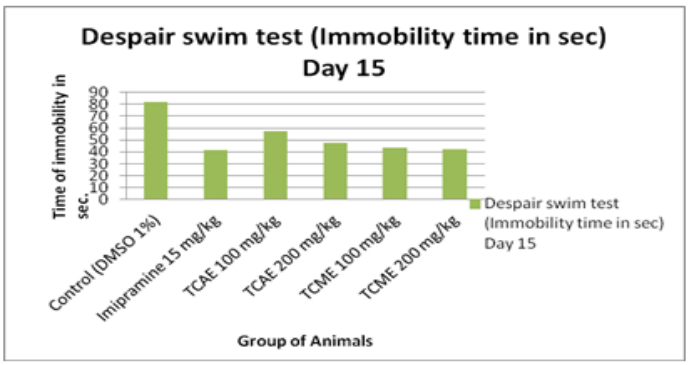

Chart 4: Despair swim day 15 test of *Terminalia Catappa Linn.leaves* extracts.

Table 8: Effect of Day 15 TCAE and TCME extracts on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)
	Day 15
Control (DMSO 1%)	81.83 ± 1.55
Imipramine 15 mg/kg	41.5 ± 1.83 ***
TCAE 100 mg/kg	57.16 ± 1.40 ***
TCAE 200 mg/kg	47.16 ± 1.70 ***#
TCME 100 mg/kg	43.66 ± 1.80 ***#
TCME 200 mg/kg	42.33 ± 1.60 ***#

Table 9: Comparative effect of TCAE and TCME on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)		
	Day 1	Day 8	Day 15
Control (DMSO 2%)	86.66 ± 1.14	83.83 ± 1.49	81.83 ± 1.55
Imipramine 15 mg/kg	44.83 ± 1.35 **	42.83 ± 1.53**	41.5 ± 1.83 **
TCAE 100 mg/kg	62.5 ± 1.60 **	60.83 ± 0.94**	57.16 ± 1.40 **
TCAE 200 mg/kg	57.33 ± 1.60 **	53.66 ± 1.85 **	47.16 ± 1.70 **#
TCME 100 mg/kg	52.33 ± 1.60 **	48.5 ± 1.47 **#	43.66 ± 1.80 **#
TCME 200 mg/kg	45.16 ± 0.94 **#	43.5 ± 1.60 **#	42.33 ± 1.60 **#

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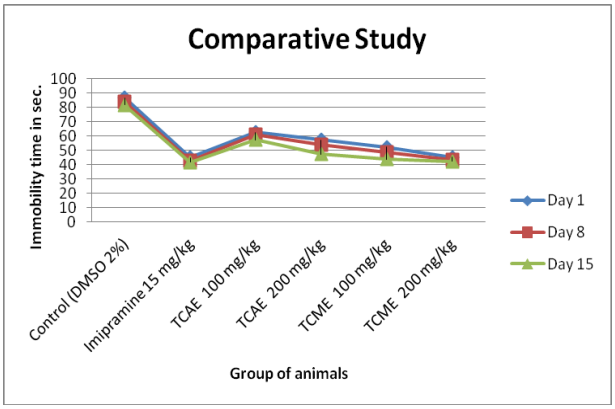

Chart 5: Comparison between all three (1st, 8th, 15th) days data time on Immobility in seconds

(N=6) Values are expressed as Mean ± SEM. Significance when compared to control group indicated with symbol*P<0.05, **P<0.001.Compared to standard group indicated with symbol #P>0.05 (NS), indicated as No significant difference when compared to standard.

When we consider average readings it was observed that the Acetone and Methanol extract show better results at 200mg/kg concentration as they reduce immobility time in swim despair test. The obtained result shows significant difference with control group and non significant difference with standard group.

X. DISCUSSION

Leaves of *Terminalia Catappa* is reported to have medicinal values including anticancer, antidiabetic, anti-inflammatory, antibacterial, antiulcer, hepatoprotective, antioxidant properties [9]. The phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, tannins, quinines were reported as the basis of therapeutic properties. The other important constituents which include punicalagin, punicalin, quercetin, tercatain, tergallagin, terflavin A, and terflavin B [10-12]. To the best of our knowledge, no scientific data regarding the antidepressant effect of *Terminalia Catappa* leaves thus the present study undertaken for comparative evaluation of *Terminalia Catappa* leaves for Petroleum ether, Acetone, Methanolic extract for antidepressant activity on Wistar rats.

In the present study we investigated the pharmacognostic characteristics of *Terminalia Catappa* leaves powder. Preliminary phytochemical study was carried out for determination of presence of phytoconstituents. Extracts shows presence of alkaloid, glycosides, tannin, carbohydrates, proteins, amino acids, flavonoids and steroids.mThe total phenolic and flavonoid as compared with the acetone and petroleum ether extract. The evaluation of antioxidant activity of *Terminalia Catappa Linn.* Leaves extract was done by using DPPH radical scavenging assay method at concentration of 25,50,75,100,125µg/ml. The ehanolic extract showed maximum percentage inhibition at 5µg/ml as compared to acetone and petroleum ether. Gallic acid, ascorbic acid, and rutin were used as standard. The result indicates that the Methanolic extract has more phytochemical constituent which is responsible for antioxidant activity.

Acute toxicity study were carried out as per literature survey by referencing LD_{50} range upto the doses of 2000mg/kg of selected plant extract at doses 100mg/kg and 200mg/kg as per OECD guidelines No.423.

Experimental Despair Swim Test model was used to test antidepressant activity of acetone and methanolic extract of plant material. The TCAE and TCME at doses 100mg/kg nd 200mg/kg and Imipramine 15mg/kg all significantly reduced immobility time compared with control DMSO 1% . Wistar Rat $p<0.05$ to $p<0.001$ and $p>0.05$ was considered non-significant (ns).Post hoc analysis Tukey's multiple comparisons test found that TCME 200mg/kg has significant difference when compared to Imipramine standard (15mg/kg) but activity more than standard in DST. *Terminalia Catappa* exhibited a slight but non-significant dose-dependent decrease in immobility. The result indicated that TC showed significant antidepressant-like effect in the DST. On day 1 the oral administration of TCAE and TCME (100mg/kg, 200mg/kg) and Imipramine (15mg/kg) p.o. shows less significant decrease in duration of immobility compared to day 8. On day 8 the oral administration of TCAE and TCME (100mg/kg, 200mg/kg) and Imipramine (15mg/kg) p.o. shows more significant decrease in duration of immobility compared to day 15. On day 15 it shows more significant decrease in immobility time in sec compared to day 1 and day 8. On day 15 TCME (200mg/kg) showed significant difference when compared to Imipramine (15mg/Kg) but is same like standard.

XI. SUMMARY AND CONCLUSION

It was planned to explore *Terminalia Catappa Linn.* Leaves part for its antidepressant potential and accordingly three extract were prepared first deffacted with petroleum ether and further extracted with acetone and methanol. These extracts were tested for its phytochemical estimation and antioxidant and antidepressant activity. Preliminary phytochemical evaluation of three extract was carried out for the determination of presence of phytoconstituents. It reveals that all three extract (i.e. Petroleum ether,Acetone, and Methanol) contain carbohydrate, glycosides, alkaloids, proteins, steroids, tannins, flavonoids. The two extract were tested for *in vivo* antidepressant activity. The obtained results showed that all the extracts showed significant effect at respective doses of 100mg/kg and 200mg/kg according to body weight when compared with standard drug imipramine. The methanolic extract at a dose of 200 mg/kg showed best result than other acetone extracts. It shows the antidepressant potential by Despair Swim

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Test (physical method) by immobility state produced by animals.

The animals are forced to swim in a restricted space from which they cannot escape are induced to a characteristics behavior of immobility. The mean immobility time for each group is observed on day 1, day 8, and day 15. From this study is concluded that the acetone and Methanol Extracts of *Terminalia Catappa Linn. leaves* showed significant Antidepressant action when compared with control group and standard Imipramine treated groups.

The different extracts of *Terminalia Catappa Linn.* Leaves shows significant antidepressant activity. The methanol extract shows more significant activity at respective doses compared to acetone extract. This is a baseline work; further investigation is needed at molecular level for determination of active constituents responsible for antidepressant activity.

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