IN-VIVO ANALGESIC ACTIVITY OF *TAMARINDUS INDICA LINN*

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Abstract: The present study investigates the *in-vivo* analgesic potential of the hydroalcoholic extract of *Tamarindus indica* L. seeds. Phytochemical screening confirmed the presence of several bioactive constituents such as alkaloids, flavonoids, phenols, tannins, saponins, and glycosides. Antioxidant activity was evaluated using DPPH radical scavenging, reducing power, and phosphomolybdenum assays, demonstrating significant antioxidant potential with IC_{50} values of 187.09 µg/mL and 160.98 µg/mL in DPPH and phosphomolybdenum assays, respectively. The total phenolic, flavonoid, and alkaloid contents were found to be 95.23 mg GAE/g, 29.46 µg QE/g, and 101 mg AE/g of extract, respectively. In vivo analgesic activity assessed via the hot plate method in Swiss albino mice showed a dose-dependent increase in reaction time, with the 400 mg/kg dose exhibiting significant central analgesic activity comparable to the standard drug pentazocine. These findings suggest that *Tamarindus indica* seed extract possesses potent antioxidant and analgesic properties, supporting its traditional medicinal use and potential therapeutic applications.

Key Words: *Tamarindus indica*, antioxidant activity, analgesic activity, DPPH assay, hot plate method, phytochemicals, flavonoids, phenols, alkaloids, natural remedies.

I. INTRODUCTION

Tamarind seeds are an agricultural byproduct that may be used to extract natural aroma components at a low cost. The cellulose-based backbone of Tamarindus indica is a branching polymer that contains galacto-xylose and xylose carrier. With a molar ratio of 3:2:1, the combination of galactose and glucose-xylose included roughly 65-72% galacto-xylose residues[1]. grows best in tropical and subtropical regions with an average temperature of about 25 C.It is said to be the perfect tree for semi-arid areas because it can withstand drought for five to six months, despite the fact that it cannot withstand extremely cold weather. Tamarind (Tamarindus indica L.) is made up of 70% kernel and 30% hard brown seed coat, with the substance containing a lot of gum (TSG), which is mostly made of a galactoxyloglucan polymer.

Tamarind is a versatile fruit, which can be used for many purposes. The unique sweet/sour flavour of the pulp is popular in cooking and flavoring. Often referred to as tamarind, it is a member of the Fabaceae family. Tamarindus indica can be eaten raw or ripe, and it can be processed to make a variety of goods. There are two primary types of tamarind: sweet and sour. Invert sugar, citric acid, oleic acid, linoleic acid, orientin, lupeol, pipecolic acid, phthalein, potassium, phenylalanine, leucine, vitamin B3, vitamin C, vitexin, campestral, β-amyrin, β-sitosterol, tannins, saponins, and glycosides are all included. Its composition lends it a variety of therapeutic applications, including the treatment of liver ailments, as an acid refrigerant, as a mild laxative, in yellow fever, as a blood tonic, in jaundice, and as a skin cleanser.

II. EXPERIMENTAL SECTION

1.Collection of Tamarindus indica seeds:
Seeds of *Tamarindus indica L.* were procured from market and coarsely powdered and stored in air tight container for further use.

Preparation of extract:
Hydroalcoholic extract of seeds of *Tamarindus indica L.* was prepared by method of maceration. In this method the powdered seeds of tamarind was macerated by using Hydroalcoholic [ethanol: water (6:4)] as solvent for 2-3 days to evaporate the Hydroalcoholic solvent to get the extract.

Phytochemical Analysis:
Hydroalcoholic extract obtained as above was subjected for the various chemical tests to identify various phytoconstituents.

ESTIMATION OF TOTAL PHENOLIC CONTENT:

The total phenolic content (TPC) for the Hydroalcoholic extract of *Tamarindus indica L.* seeds were determined by using the Folin-Ciocalteu phenol reagent method[2].

Briefly, 1.0 ml of the extract at various concentrations was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2.5 ml of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for about half an hour (30min). The absorbance was read at 750 nm spectrophotometer. The total phenol content was expressed as gallic acid equivalents in mg/gm of the extract.

ESTIMATION OF TOTAL FLAVONOID CONTENT:

The flavonoid content (TFC) Hydroalcoholic extracts of *Tamarindus indica L.* seeds were screened using aluminium chloride colorimetric method[3]. Quercetin was used as a reference standard for plotting a calibration curve.

TFC is determined by the following procedure 0.5ml of the extract at various concentrations was mixed with 3 ml of 95% methanol, 0.1ml of 10% (weight/volume) aluminium chloride, 0.1ml of 1M potassium acetate, and 2.8 ml of distilled water. Let the reaction mixture stand at room temp for about 30 min and absorbance was read at 415 nm against the blank sample. A calibration curve was generated using quercetin in methanol. The flavonoid content was expressed as quercetin equivalents in mg/g of the extract.

ESTIMATION OF TOTAL ALKALOID CONTENT:

The total alkaloid content of *Tamarindus indica L.* extracts was determined by a slightly modified Bromocresol green (BCG) method. Atropine was used as the reference standard for plotting the calibration curve.

sample preparation:

Dissolve 1 mg of extract in 1ml of 2N HCl and add 1 ml of Dimethyl sulfoxide then filter it. From the above solution take 1 ml and transfer it into separating funnel. Add 5ml of bromo cresol green solution and add 5 ml of phosphate buffer then the mixture was shaken with 1ml, 2ml, 3ml, 4ml of chloroform. Collect the chloroform layer after every shaking, then make up the volume up to 10ml with chloroform in 10ml volumetric flask. Measure the absorbance at 470 nm.

IN-VITRO ANTIOXIDANT POTENTIAL:

DPPH Antiradical capacity:

The free radical potential of Hydroalcoholic extract of *Tamarindus Indica L.* seeds was determined spectrophotometrically[4]. A stock solution of DPPH was prepared by dissolving 33mg of DPPH in 1 litre of methanol.

Five different concentrations of Hydroalcoholic extract of Tamarindus indica L. seeds (100, 200, 400, and 800 and 1000 µg/ml) were mixed with 100 µl of DPPH radical solution in a 96-well microplate and incubated for 20 min at room temperature. The resultant mixture was read spectrophotometrically at 517 nm against a methanol blank and the following equation was used to calculate the % inhibition of each extract: % inhibition (Ao-As)/(40) ×100 Where, Ae is the absorbance of the control, and As is the absorbance of the test sample. The IC represented the concentration of the extract that inhibited 50% of radical.

Reducing power Determination:

The reducing ability of a compound generally depends on the presence of reductants which exhibits antioxidant potential by breaking the free radical chain, donating a hydrogen atom.[5] Hydroalcoholic extract of *Tamarindus indica L.* seeds (0.75ml.) at various concentrations sere mixed with 0.75 ml of phosphate buffer (02 mole, pl1 5.6) and 0.75 ml. of potassium tricyanide $K_3Fe(CN)$ (1%w/v), followed by incubating at 50°C for 20 mins. The reaction was stopped by adding 2.5ml. of 10% (w/v) trichloroacetic acid followed by centrifugation at 3000 rpm for 10min. Finally, 1.5ml. of the upper layer was mixed with 15ml of distilled water and 0.5mL of $FeCl_3$ (0.1%) and the absorbance was creased at 700 nm. Higher the absorbance of reaction mixture indicated greater the reducing power. Ascorbic acid is seed as reference compound.

Total Antioxidant Activity:

Total antioxidant activity was estimated by phosphomolybdenum assay[6]

Hydroalcoholic extract of *Tamarindus indica L.* in different concentration ranging from 100 µg/ml to

500 µg/ml were added to each test tube individually containing Jal of distilled water and 1ml of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695nm. Mean values from independent samples were calculated for each extract ascorbic acid was used as positive reference standard.

Total antioxidant = OD of test × concentration of standard in ug × made up volume of sample

EVALUATION OF IN-VIVO ANALGESIC ACTIVITY:

Animals:

This study's an ethical approval was acquired from the "Institutional Animal Ethical Committee" with an Approval no: MRCP/CPCSEA/IAEC/2025/1/12. Albino rats with average body weight from 150 to 250 g were utilized for conducting this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur (V), Shameerpet, RR dist. Polypropylene cages were used for housing rats as well as standard conditions (12h dark and light cycles at 35-60 % humidity and 25±3°C) were used for their maintenance. Standard tap water and pellet feed were allowed adlibitum.

IN-VIVO ANALGESIC ACTIVITY:

Analgesic Activity was screened by Hot plate method. Wistar albino rats were treated according to the method described by Eddy and Leimback, 1953 [7]. Rats were screened by placing them on hot plate (UGO Basile, Italy. Model No. DS-37) maintained at55 ± 1 °C and the reaction time was recorded in seconds. The time for paw licking or jumping on the hot plate was considered as are action time. The responses were recorded before and after 30, 60, 90, 120, 150 and 180 min after the administration of Hydroalcoholic extract of *Tamarindus indica* seed (at different doses) and Pentazocine. A cut-off time of 15s was used to avoid injury to the animals.
The Rats were divided into five groups of 6 Rats each.
Group 1: - Vehicle control (2% Tween 80).
Group 2: - Standard (Pentazocine 5 mg/kg s.c.).
Group 3: - Hydroalcoholic extract of Tamarindus indica seed (100 mg/kg, p.o.)
Group 4: - Hydroalcoholic extract of Tamarindus indica seed (200 mg/kg, p.o.)
Group 5: - Hydroalcoholic extract of Tamarindus indica seed (400 mg/kg, p.o.)

IV.RESULTS AND DISSCUSSION:

Phytoconstituents were identified by performing qualitative analysis and "+" indicates presence and "-" indicates absence of phytoconstituents (Table 1).

Table 1: Phytochemical screening of Hydroalcoholic extract of *Tamarindus indica L.*seeds

Phytochemicals	AETI
Carbohydrates	+
Proteins	-
Alkaloids	+
Steroids	+
Glycosides	+
Flavonoids	+
Fixed oils	_
Saponins	+
Phenols	+
Tannins	+

ESTIMATION OF TOTAL PHENOLIC CONTENT:

Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. In the current work, phenolic content of Hydroalcoholic extract of *Tamarindus indica L.* seeds was measured and was found to be 95.233 mg. The results are described as Gallic acid equivalents (GAE) (Figure 1).

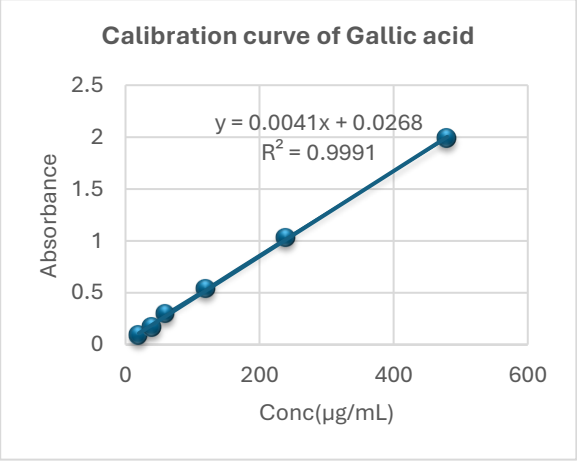

Fig 1: Calibration curve of Gallic acid

ESTIMATION OF TOTAL FLAVONOID CONTENT:

Flavonoids have gained recent attention because of their broad biological and pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti -inflammatory as well as antitumor activities but the best described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. Total flavonoid content in Hydroalcoholic extract of *Tamarindus indica L.* seeds was calculated in terms of Quercetin equivalents in mg per gm of the extract. using aluminium chloride colorimetric method. In the current study, the total flavonoid content was measured and was found to be 29.46 µg. The flavonoid content was expressed as quercetin equivalents in microgram per gram of the extract (Figure 2)

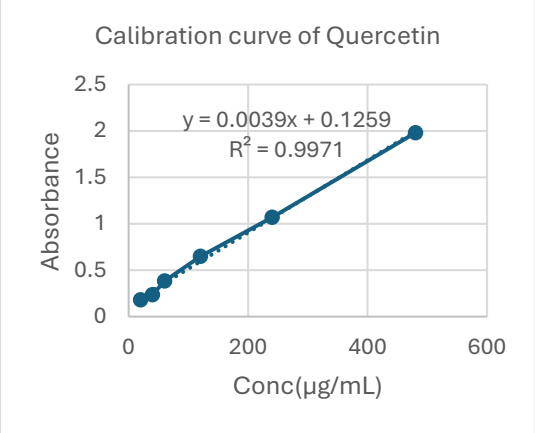

Fig 2: Calibration curve of Quercetin

ESTIMATION OF TOTAL ALKALOID CONTENT:

The determination of total alkaloids using a visible spectrophotometric method with Bromocresol Green (BCG) is a simple and sensitive technique that requires no special equipment. BCG can react with certain alkaloids, i.e., the ones that have nitrogen inside their structure, but not with amine and amide alkaloids. The reaction of alkaloids with BCG forms a yellow-coloured product. (Figure 3) Total alkaloidal content of Hydroalcoholic extract of *Tamarindus indica L.* seeds was calculated in terms of Atropine equivalents in mg per gm of the extract. Total alkaloid content was found to be 101mg.

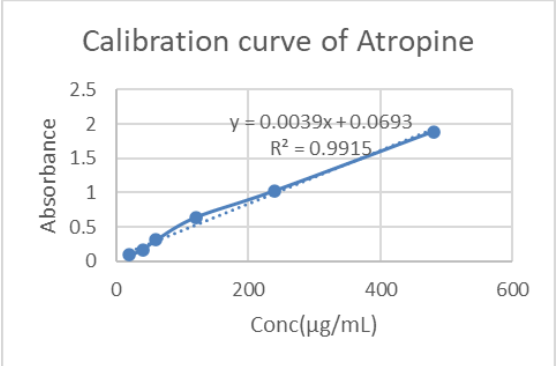

Fig 3: Calibration curve of Atropine

IN-VITRO ANTIOXIDANT POTENTIAL:

DPPH Antiradical capacity:

DPPH is a relatively stable free radical and the assay determines the ability of Hydroalcoholic extract of *Tamarindus indica L.* seeds to reduce DPPH free radicals to the corresponding hydrazine by converting the unpaired electrons to paired ones Antioxidant can act by converting the unpaired electron to paired one. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance at 517nm and also for visible deep purple colour. (Figure 4) and the IC50 value was calculated as 187.09 µg/mL using GraphPad Prism.8.6.1

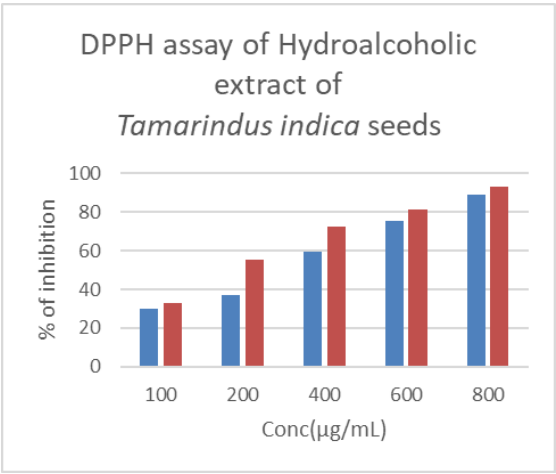

Fig 4: DPPH assay of hydroalcoholic extract of *Tamarindus indica* seeds

Reducing power Determination:

Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions are reduced to ferrous ions, identified by colour

change from yellow to bluish green. The results for ferric reducing power activity of Hydroalcoholic extract of *Tamarindus indica L.* seeds in comparison with ascorbic acid are reported in (Figure 5). Reducing power potential of extracts increase with the dose, however the extract exhibited low reducing power than that of ascorbic acid.

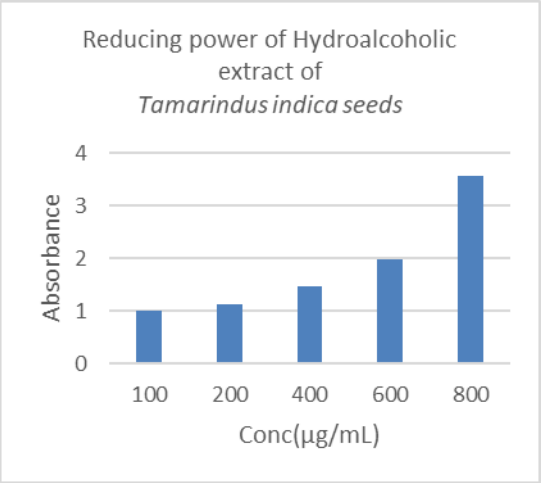

Fig 5: Reducing power of hydroalcoholic extract of *Tamarindus indica* seeds

Total Antioxidant Activity:

The seed extract various concentrations were assayed for their antioxidant potency by the formation of green phosphomolybdenum complex. The total anti-oxidant capacity was measured by taking ascorbic acid as standard. IC_{50} was calculated by using GraphPad Prism 8.3.1 and was 160.98µg/ml. (Figure 6)

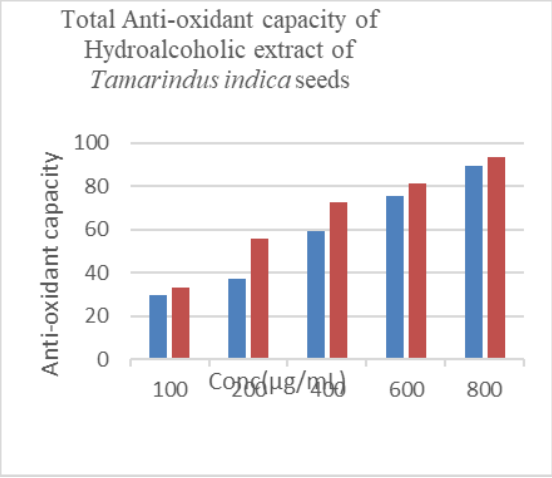

Figure 6: Total anti-oxidant assay of Hydroalcoholic extract of *Tamarindus indica L.* seeds

IN-VIVO ANALGESIC ACTIVITY:

Group-wise observations:

In Group 1, Reaction time remained constant and low (~3-4 seconds), indicating no analgesic effect.

Whereas, Group 2 animals Showed significantly increased reaction times across all time intervals. Peak effect observed at 60-90 min with reaction time up to 12-14 seconds.

Group 3 animals showed Mild increase in reaction time (~5-7 seconds). Peak at 90min, not statistically significant compared to control.

Moderate analgesic activity observed in Group 4 animals treated with Tamarindus indica extract (200mg/kg, p.o.) Reaction time was increased to 7-9 seconds, with peak effect around 90-120 min.

Tamarindus indica extract 400mg/kg, p.o. treated Group 5 animals Significant increase in reaction time (10-12 seconds). Effect was dose-dependent and statistically significant compared to control ($p<0.05$).

Fig 7: Administration of drug to the Rats

The reaction time for paw licking or jumping response on the hot plate was recorded at different intervals (30, 60, 90, 120 150, and 180 minutes). The results are expressed as mean reaction time (in seconds) ± SEM for each group.

The Hydroalcoholic extract of *Tamarindus indica* seed exhibits significant analgesic activity in a dose-dependent manner, with the 400mg/kg dose approaching the efficacy of the standard drug pentazocine.

Group	Treatment	Dose	Reaction time (seconds, Mean ± SEM)	Peak effect time (min)	Significance
1	control	-	3.2 ± 0.4	-	-
2	Pentazocine	5mg/kg s.c.	12.8 ± 0.6	60-90	P< 0.001 vs control
3	*T. indica* extract	100mg/kg p.o.	6.1 ± 0.5	90	Not significant
4	*T. indica* extract	200mg/kg p.o.	8.4 ± 0.6	90-120	P< 0.05 vs control
5	*T. indica* extract	400mg/kg p.o.	11.2 ± 0.5	120	P< 0.01 vs control

Table 2: Effect of Hydroalcoholic extract of *Tamarindus indica* seed on reaction time in hot plate method

V.CONCLUSION AND SUMMARY

The hydroalcoholic extract of *Tamarindus indica L.* seeds exhibits significant central analgesic activity in a dose-dependent manner. The 400 mg/kg dose showed notable enhancement in pain threshold, comparable to standard drug Pentazocine, confirming the extract's efficacy in managing pain through central mechanisms. The presence of phytochemicals such as tannins, alkaloids, and phenolics may contribute to its pharmacological effects. Moreover, the antioxidant activity reinforces its therapeutic potential by possibly mitigating oxidative stress-associated pain mechanisms. These results validate the ethnomedicinal use of *Tamarindus indica* and encourage further studies to isolate active constituents and explore its mechanism of action.

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