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AN INVITRO STUDY ON ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITIES OF ACETONE EXTRACT FROM FRUIT OF TERMINALIA CATAPPA

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Abstract

Experimental studies explored the antidiabetic and anti-inflammatory effect of acetone extract from the fruits of *Terminalia catappa* in various invitro models, but previously no study was conducted to establish the antidiabetic, and anti-inflammatory potentiality of acetone extract from the fruits of *Terminalia catappa*. The present study investigate the antidiabetic and anti-inflammatory effects of acetone extract from the fruits of *Terminalia catappa* with the intention to find the drug for diabetes and thrombosis management from natural sources. The hypoglycemic effect of the decoction was tested in glucose uptake, inhibition of alpha amylase and alpha-glucosidase effects. The anti-inflammatory activity was assessed by using albumin denaturation, inhibition of lipoxygenase and the results were compared with standard diclofenac sodium. In the present research, the antidiabetic effect of acetone extract from the fruits of *Terminalia catappa* reduces the glucose uptake, inhibition of alpha amylase and alpha-glucosidase effects at a dose and time dependent manner. It was observed that the plant possess significant antidiabetic, and anti-inflammatory effect of acetone extract from the fruits of *Terminalia catappa*.

Key words: Acetone extract; fruits of *Terminalia catappa ;* antidiabetic; anti-inflammatory

INTRODUCTION

Plants, which are sources of phytochemicals with strong antioxidant activity, have attracted a great deal of attention in recent years. Antioxidants, which inhibit the oxidation of organic molecules, are very important, not only for food preservation, but also for the defence of living systems against oxidative stress (Tsay and Agrawal, 2005). These plants contain substances that can be used for therapeutic purposes, of which are precursors for the synthesis of drugs. A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems; as well as the sexual organs, the skin, vision, hearing and taste (Bailey and Day, 1993).

Phenolic antioxidants interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes. Diabetes mellitus (DM) also known as simply diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (World Health Organization, 2021). In this condition hyperglycemia, or the accumulation of glucose (sugar) occurs in the bloodstream. This high blood

sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications involve diabetic ketoacidosis and non ketotic hyperosmolar coma. According to International Diabetes Federation, DM affects nearly 10% of the world population many drugs are currently available for the management of diabetes but most of them are expensive and have potential side effects e.g. Adjunctive exenatide causes hypoglycemia and obesity (David et al., 2008). That's why; screening of plants for hypoglycemic activity will be of enormous implication in this circumstance.

Foods influence inflammation in multiple ways. Some foods, including trans-fats and charred foods, have pro-oxidant effects (Brighenti *et al.,* 2005). Foods with high glyemic index or glycemic load values are more pro-inflammatory (Diabetes mellitus Type II is preceded by elevations in inflammatory markers). In addition, many food compounds directly alter specific biochemical pathways, the most researched of these being essential fatty acids (EFAs). The body is unable to synthesize omega-6 and omega-3 fatty acids, so they must be obtained in the diet (Simopoulos 1999).Omega-6 fatty acids are overall pro-inflammatory. Omega-3 fatty acids decrease inflammation by decreasing the metabolism of arachidonic acid into inflammatory prostaglandins and leukotrienes. Common omega-3 fatty acids include alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexanoic acid (DHA). Since the Paleolithic era, the ratio of omega-6 to omega-3 fats in the human diet has steadily increased, from approximately 1-2:1 to over 25:1. This rise seems to correlate with the rise of many chronic illnesses (Shinde et al.,1999).

Fruits of *Terminalia catappa* is one such plant assumed to control diabetic; inflammatory diseases which belongs to the family Combretaceae. The mature plant that grows to about 1.3 m with leaves that are simple anlternate. Its flowers are hermaphrodite, having both male and female organs appear seperatly. Its flowers are regular, epygynous. Medicinal uses of *Terminalia catappa* Fruits include the treatment of stomach ache, ear pain and sepsis, diphtheria, vomiting, promotion of labor at the end of pregnancy, and snake bite. These herbal treatments applied by traditional healers and rural populations need to be documented scientifically for their safety and efficacy, and feedback given to the community. Therefore, the present study was conducted to determine the in vitro antidiabetic and anti-inflammatory activity of fruits of *Terminalia catappa*,

MATERIALS AND METHODS

PLANT MATERIALS

The Fruits of *Terminalia catappa* were collected from Government siddha medical college, herbal garden, Arumbakkam, Chennai, Tamilnadu, during Jan 2023 and it was taxonomically identified and authenticated as leaves of *Andrographis echioides* by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamilnadu. A voucher specimen was deposited in the herbarium for future reference (Ref.No. MB/2021/Ceasal-389).

PHYTOCHEMICAL ANALYSIS

The aqueous fruit extract of *Terminalia catappa* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973; Trease and Evans 1983).

TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of acetone extract from the fruits of Fruits of *Terminalia catappa* was determined using the method by Gutfinger (1981). The methanol extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400X *g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (ELICO (SL150) UV–Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a catechol equivalent.

ESTIMATION OF FLAVANOID

1ml aliquot of acetone extract from the fruits of *Terminalia catappa* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml 0f 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Delcour and de Varebeke, 1985). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

THIN LAYER CHROMATOGRAPHY PROFILE

The acetone extract from the fruits of *Terminalia catappa* were loaded on to pre coated TLC (60 F_2 54) and it was developed using solvent system in the ratio of Petroleum ether, Chloroform and methanol (1:0.5:0.1, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F_{254} (10x20 cm, 0.2mmlayer). Visible and the non-visible spot given and it is fluorescent with UV light at 360nm and 240nm.

GLUCOSE UPTAKE IN YEAST CELLS

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (25-100 µg/mL) were added to 1mL of glucose solution (25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula: Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

INHIBITION OF α-AMYLASE ACTIVITY

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by (Hamdan and Fatimai 2010) and later employed by others for determination of amylase activity in plant extracts with some modifications. In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of acetone extract from the fruits of *Terminalia catappa* of different concentration such as 25, 50, 75 and 100 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water).

Inhibition of alpha- Amylase (%) = Abs sample – Abs control × 100

INHIBITORY ACTIVITY OF α-GLUCOSIDASE

The α-glucosidase inhibitory activity was assessed by the standard method followed by Dong et al. (2012). Briefly, a volume of 60µl of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37 ºC for 20 min. After pre-incubation, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 ºC for another 20 min. Then the reaction was stopped by adding 160 µl of 0.2 M $NaCO_3$ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

Where, ACO is absorbance of the control and at is absorbance of the sample the concentration of inhibitors required for inhibiting 50% of the α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

ANTI-LIPOXYGENASE ACTIVITY

Anti-lipoxygenase activity was studied with minor modifications, using linoleic acid as substrate and lipoxidase as an enzyme (Shinde et al., 2012). Test samples were dissolved in 2M borate buffer pH 9.0 (0.25 ml) and (0.25 ml) lipoxidase enzyme solution (20,000 U/ml). The reaction mixture was incubated for 5 min at 25°C. Then, 0.6 mM lenoleic acid solution (1.0 ml) was added. The reaction mixture was vortexed, and absorbance was measured at 234 nm. Diclofenac sodium as a reference was used. The percent inhibition was calculated from the following equation:

%inhibition = Abs control -Abs sampleAbs control×100

INHIBITION OF ALBUMIN DENATURATION

The anti-inflammatory activity of acetone extract from the fruits of *Terminalia catappa* were studied according to the protocol of Mizushima et al. with some modifications. Inhibition of albumin denaturation was done according to the protocol. The reaction mixture consists of an equal volume of test acetone extract from the fruits of *Terminalia catappa* of different concentrations (25-100 µg/ml) and 1% aqueous solution of bovine albumin (Fraction V). The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min. The absorbance was measured after cooling the samples at room temperature. The turbidity formed was measured at 660 nm using ultraviolet (UV)-visible spectrophotometer . The percentage inhibition of protein denaturation was calculated as follows:

%inhibition = Abs control -Abs sampleAbs control×100

RESULT AND DISCUSSION

PHYTOCHEMICAL SCREENING

The phytochemical screening of aqueous leaf extract from the fruits of *Terminalia catappa* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1 and Fig-2).

Table-1. Phytochemical screening of aqueous leaf extract from the fruits of *Terminalia catappa*

Sl. No.	**Phytochemical Constituents**	**Observation**	**Aqueous leaf extract from the fruits of *Terminalia catappa***
1	**Alkaloids** -Dragendorff's Test -Mayers test	Orange / red precipitate Yellow or white precipitate	+ +
2.	**Flavonoids** -Alkalai Reagent -Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	**Glycosides** Keller-Killiani test	Reddish brown colour ring formed	-
4.	**Tannin** -$FeCl_3$ test	Blue black coloration	-
5.	**Saponins** -Frothing test	Foam	+

6.	**Terpenoids** *-Salkowski test*	Dark reddish brown color in interface	+
7.	**Polyphenols** -Ferrozine test	Raddish blue	+
8.	**Anthocyanin test Ammonia**	Ammonia layer yellow in color	-

+ indicate positive result; -- Indicate negative result

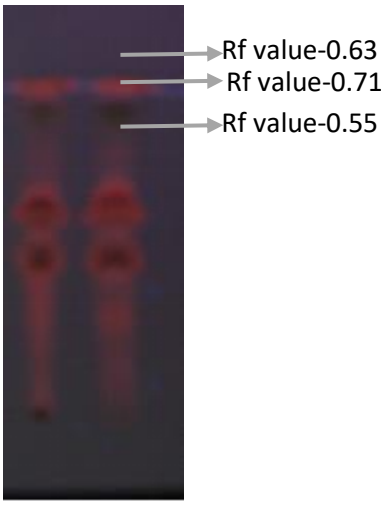
TOTAL PHENOLIC AND FLAVONOID CONTENT

The total polyphenol content, antioxidant activity and flavonoid content was determined by folin-ciocalteau method, phosphomolybdenum assay and aluminium chloride colorimetric technique respectively as stated in material and methods. In this context, the preliminary experiments revealed that acetone extract was the best solvent for the extraction of phenolics from the acetone extract from the fruits of *Terminalia catappa* at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields acetone extract from the fruits of *Terminalia catappa* ranged from 59.23% (w/w) and 55.69 % (w/w). Therefore, the total phenolic and flavonoid contents were reported as catechin and rutin equivalents respectively.

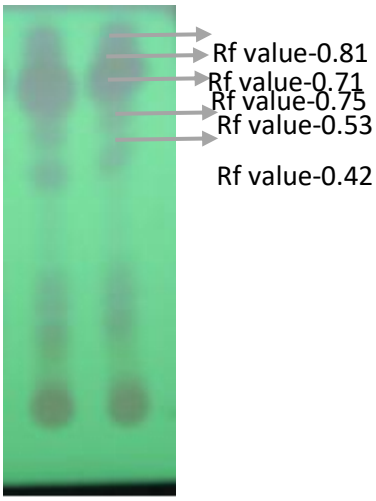
TLC PROFILE

The acetone extract from the fruits of *Terminalia catappa* loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of Toulene, dioxin and acetic acid in the ratio of 9.5:2.5:0.4. The developed plate was viewed under UV 240nm and 360nm (Fig-1).

Fig-1. TLC profile of acetone extract from the fruits of *Terminalia catappa*



TLC plate viewed under UV light 240 nm



TLC plate viewed under UV light 360 nm

GLUCOSE UPTAKE IN YEAST CELLS OF ACETONE EXTRACT FROM THE FRUITS OF *TERMINALIA CATAPPA*

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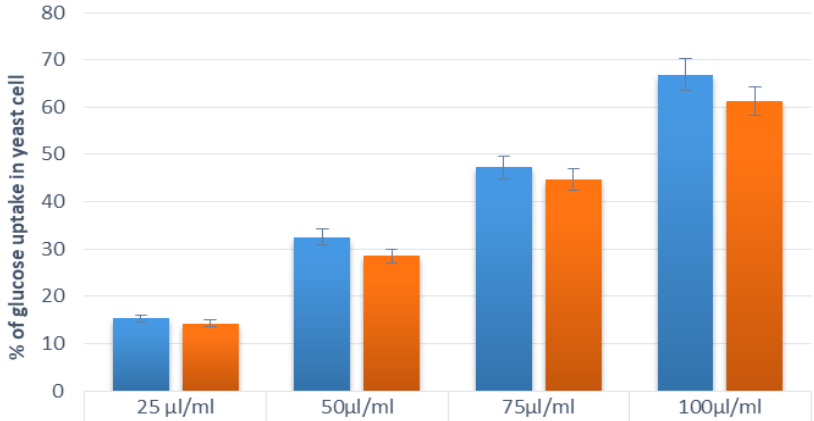
The rate of glucose transport across cell membrane in yeast cells system is presented in Graph- 1. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the three glucose concentrations. The acetone extract from the fruits of *Terminalia catappa* exhibited significantly higher activity (87.32%) at all concentrations than standard (72.38%). However the highest uptake of glucose was seen in 25mM Glucose concentration. The result showed the lower uptake of glucose by the yeast cells which conformed the highest activity.

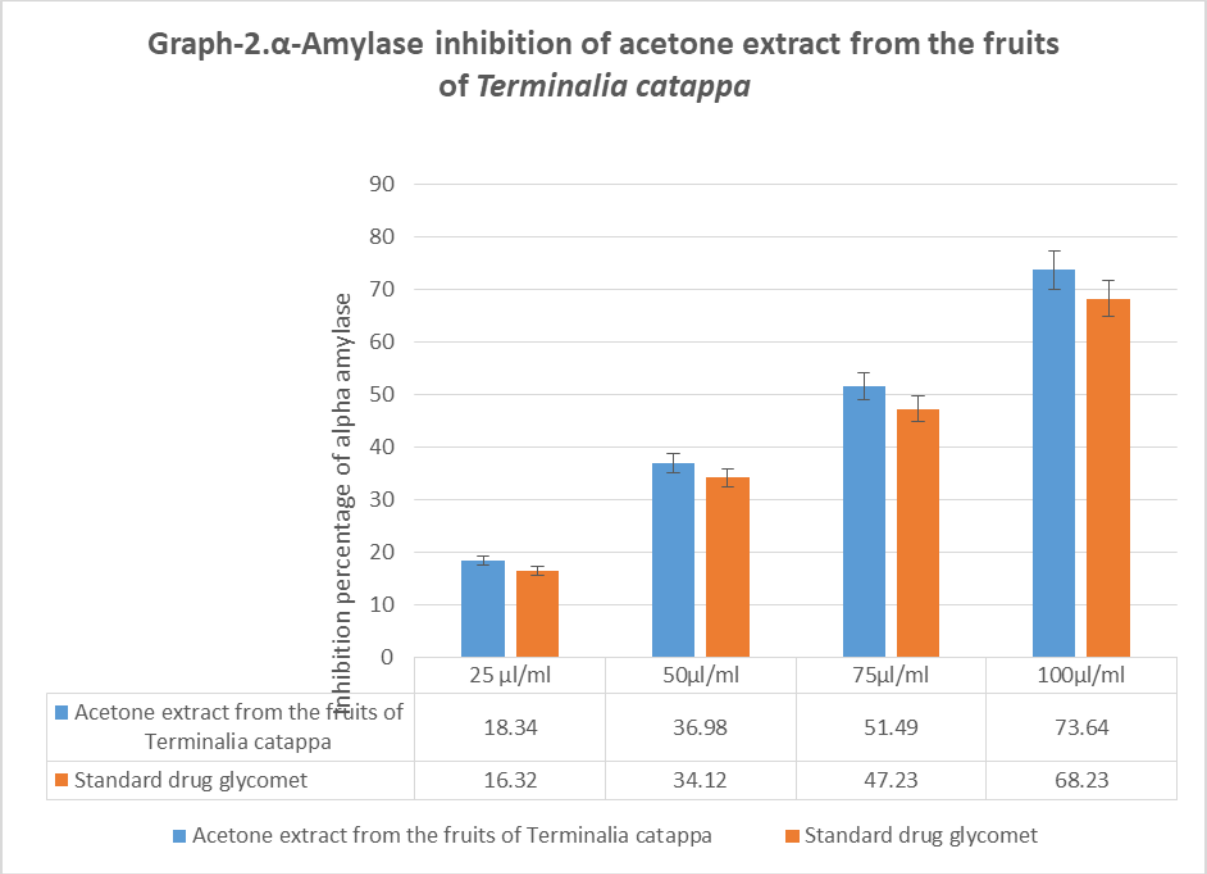
Graph-1. Glucose uptake in yeast cells of acetone extract from the fruits of *Terminalia catappa*



	25 µl/ml	50µl/ml	75µl/ml	100µl/ml
Acetone extract from the fruits of Terminalia catappa	15.32	32.56	47.23	66.89
Standard drug glycomet	14.25	28.56	44.76	61.23

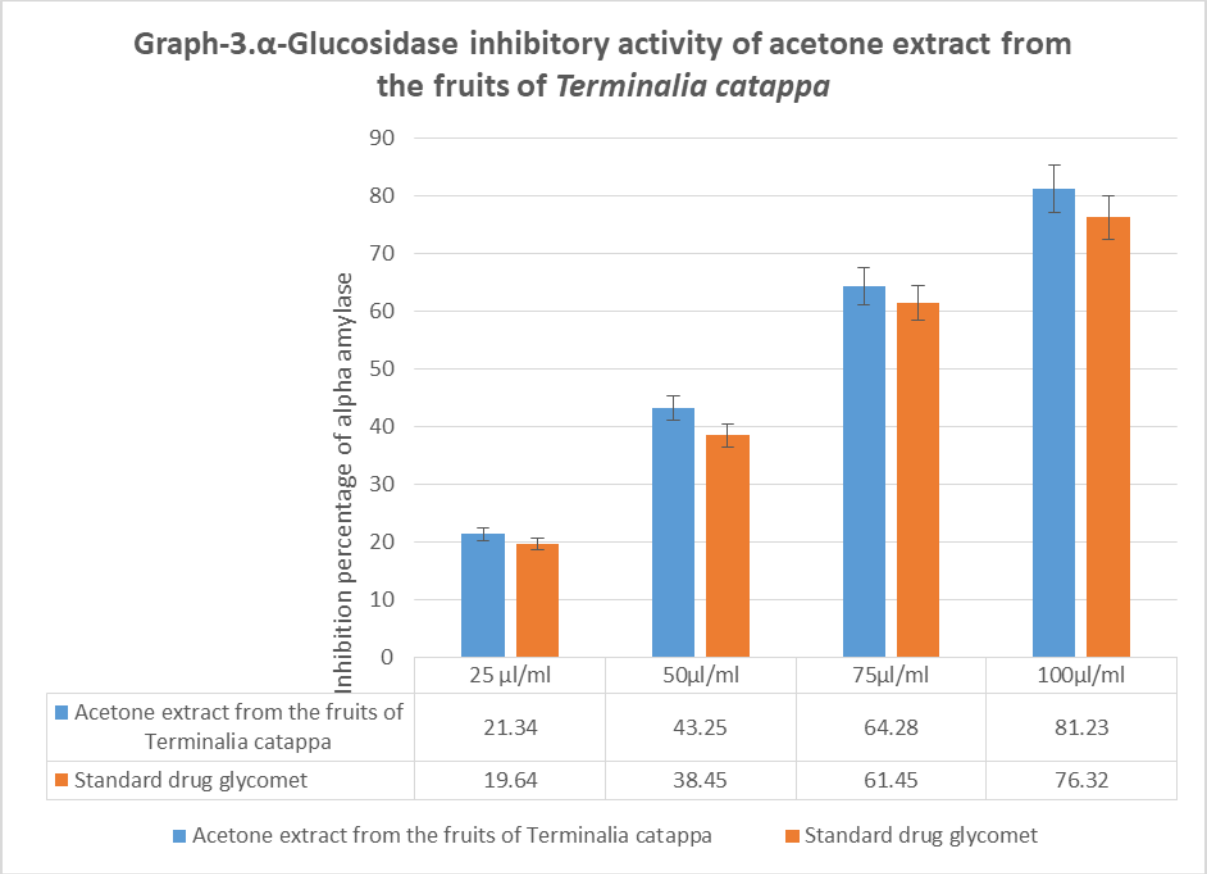
α-AMYLASE INHIBITION OF ACETONE EXTRACT FROM THE FRUITS OF *TERMINALIA CATAPPA*

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha-bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. In present experimental study it was observed that acetone extract from the fruits of *Terminalia catappa* demonstrated inhibition of alpha amylase. But the result of acetone extract from the leaves of acetone extract from the fruits of *Terminalia catappa* significant inhibition of alpha amylase activity (793.32%) as compared to standard drug glycomet (74.38%) (Graph-2). Multiple mechanisms, due to many phytoconstituents, were documented for the antidiabetic activity of medicinal plants. Therefore, documenting the efficacy of antidiabetic medicinal plants has been increased, and their characterizations of chemical constituents are focused in drug discovery programmes to bring a better lead molecule to treat diabetes (Tiwari and Rao, 2002).



α-GLUCOSIDASE INHIBITORY ACTIVITY OF ACETONE EXTRACT FROM THE FRUITS OF *TERMINALIA CATAPPA*

The results of *in-vitro* α-glucosidase inhibitory study are showed in Graph-3. The acetone extract from the fruits of *Terminalia catappa* showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 µl/ml tested showed a maximum inhibition of nearly 73.34% acetone extract from the fruits of *Terminalia catappa* seems to be less potent in α-glucosidase inhibitory potential compared to glycomet (70.33%). It may be that α-glucosidase is more sensitive towards glycomet with the concentration required for 50% inhibition (IC_{50}) found to be 66.38µg/ml. The key enzymes for carbohydrate metabolism in the small intestine are pancreatic *α*-amylase and *α*-glucosidase which convert consumed polysaccharides to monosaccharides. This enzyme action causes postprandial blood glucose level elevation due to absorption of formed glucose from polysaccharides in the small intestine. New drugs or formulations which are devoid of the above side effects will improve the compliance in type 2 diabetic patients (Bhandari et al., 2006).



LIPOXYGENASE INHIBITION ACTIVITY OF ACETONE EXTRACT FROM THE FRUITS OF *TERMINALIA CATAPPA*

The inhibition of LOX using linoleic acid as substrate was determined for the anti-inflammatory activity in the acetone extract from the fruits of *Terminalia catappa*. The acetone extract from the fruits of *Terminalia catappa* at 100µl/ml concentration exhibited more inhibition than the other concentration. The inhibition percentage was above 72.32% at 100µl/ml (Table-2). The standard diclofenac sodium was showed 67.23% inhibition at 100 µg/mL. The acetone extract from the fruits of *Terminalia catappa* was showed higher inhibition activity than positive control. Lipoxygenase catalyzes the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-1, 4-pentadiene system. This reaction originates unsaturated fatty acid hydroperoxides. These products are further converted into others that play a key role in inflammatory processes. Hence, compounds which are able to inhibit that enzyme can be considered as antioxidants and possessing anti-inflammatory properties (Palmieri et al., 2011).

Table-2. Inhibition activity of Lipoxygenase of acetone extract from the fruits of *Terminalia catappa*

Flavonoid Concentration	**Acetone extract from the fruits of *Terminalia catappa***	**Standard Diclofenac sodium**
25 µl/ml	22.34±2.67	19.34±3.14
50 µl/ml	39.64±2.18	35.64±1.78
75 µl/ml	58.34±1.36	53.21±2.89

100 µl/ml	72.32±1.56	67.23±1.23
EC_{50} Vlaue	64.23±1.59	73.64±1.75

Results are expressed as percentage inhibited Lipoxygenase with respect to control. Each value represents the mean+SD of five experiments

INHIBITION OF PROTEIN DENATURATION OF ACETONE EXTRACT FROM THE FRUITS OF *TERMINALIA CATAPPA*

Examination of acetone extract from the fruits of *Terminalia catappa* of momentous activity on inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that alkaloid extract is proficient of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. The maximum percentage inhibition of protein denaturation was observed in acetone extract from the fruits of *Terminalia catappa* 74.28% at 100µg/ml which was close to the percentage of inhibition of diclofenac sodium (71.32 %) (Table-3).

Table-3. Inhibition activity of protein denaturation of acetone extract from the fruits of *Terminalia catappa*

Different concentration	**Acetone extract from the fruits of *Terminalia catappa***	**Standard Diclofenac sodium**
25 µl/ml	20.16±1.23	18.32±0.28
50 µl/ml	38.64±1.39	35.64±1.67
75 µl/ml	55.24±2.56	51.24±1.35
100 µl/ml	74.28±1.34	71.23±1.26
EC_{50} Vlaue	61.24±1.49	66.37±2.64

Results are expressed as percentage inhibited inhibition of protein denaturation with respect to control. Each value represents the mean+SD of five experiments

CONCLUSION

Acetone extract from the fruits of *Terminalia catappa* has potential antidiabetic and anti-inflammatory activity in invitro model. In vitro study results scientifically supported acetone extract from the fruits of *Terminalia catappa*. Further, acetone extract from the fruits of *Terminalia catappa* contains active biomarkers which may possibly be responsible for the antidiabetic activity. In this studies acetone extract from the fruits of *Terminalia catappa* indicate that anti-inflammatory activity is probably due to enzymatic inhibitors.

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