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An *In vitro* study on antidiabetic and anti-inflammatory activities of commonly used green leaf of *Gynandrophis pentaphylla*

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Abstract
Experimental studies investigated the antidiabetic and anti-inflammatory effect of acetone extract from the leaves of *Gynandrophis pentaphylla* in various invitro models, but no study was conducted to establish the antidiabetic and anti-inflammatory potential of *G. pentaphylla* acetone extract. The current study investigates the anti-diabetic and anti-inflammatory effects of acetone extract from the leaves of *G. pentaphylla* in order to find a medication for diabetes and thrombosis administration from natural sources. The extracts' hypoglycemic impact was evaluated in terms of glucose take-up, alpha amylase inhibition, and alpha-glucosidase effect. The anti-inflammatory activity was assessed using albumin denaturation and lipoxygenase inhibition, and the results were compared to standard diclofenac sodium. The anti-diabetic effect of acetone extracted from the clears out of *G. pentaphylla* reduces glucose take-up, inhibition of alpha amylase, and inhibition of alpha-glucosidase impacts at a dosage and time. It was observed that the plant have noteworthy antidiabetic, and anti-inflammatory effect of acetone extract from the leaves of *G. pentaphylla*.

Keywords: acetone extract, *Gynandrophis pentaphylla*, antidiabetic, anti-inflammatory

Introduction

Plant seeds and fruits continue to be an important therapeutic aid in the treatment of human ailments. Over the last 2500 years, exceptionally strong traditional frameworks of medicine such as Siddha, Ayurveda, and the Unani have been born and practised, primarily on the eastern continent. These traditions are still alive and well, as approximately 80% of people in developing countries rely on pharmaceutical frameworks for their basic health care needs (Tsay and Agrawal, 2005) [13]. These plants contain substances that can be used for beneficial purposes, which are precursors to the drug combination. A small amount of research has been done on a few medicinal herbs, and they have been found to have beneficial effects on the nervous, circulatory, respiratory, digestive, and urinary systems, as well as the sexual organs, skin, vision, hearing, and taste (Bailey and Day, 1993) [1].

Diabetes mellitus (DM), also known as, is a group of metabolic diseases characterised by high blood sugar levels over an extended period of time (World Wellbeing Organization, 2021). Hyperglycemia, or the accumulation of glucose (sugar), occurs within the circulatory system in this condition. This high blood sugar level causes symptoms such as frequent urination, increased thirst, and increased hunger. Diabetes, if left untreated, can lead to a slew of complications. Diabetic ketoacidosis and nonketotic hyperosmolar coma are examples of acute complications. According to the Universal Diabetes Alliance, diabetes affects about 10% of the world's population. Many drugs are now available for the treatment of diabetes, but the majority of them are expensive and have potential side effects, for example. Adjunctive exenatide results in hypoglycemia and weight gain (David *et al*., 2008) [5]. As a result, screening plants for hypoglycemic action will be a huge help in this situation. Type II diabetes, which is non-insulin dependent, typically develops in adults over the age of 40. It has now been established that diabetes-related chronic hyperglycemia causes long-term damage, dysfunction, and eventually failure of organs, particularly the eyes, kidneys, nerves, heart, and blood vessels (Huang *et al*., 2005) [6]. It has a negative impact on the carbohydrate, lipid, and protein digestion systems, resulting in severe hyperglycemia and lipid profile abnormalities. These result in the development of secondary complications such as polyurea, polyphasia, ketosis, retinopathy, and cardiovascular disease (Kumar and Clark, 2002). Diabetes and its complications continue to be a major health issue around the world, affecting about 10% of the global population and being regarded as a major cause of high financial misfortune, which can obstruct countries' development (Mahabir and Gulliford, 1997) [7]

Various foods have different effects on aggravation. Some foods, such as trans-fats and burned foods, contain pro-oxidant properties (Brighenti *et al*., 2005). Foods with a long glyemic list or a high glycemic stack are more pro-inflammatory. Furthermore, a variety of food ingredients directly alter specific metabolic pathways, the first of which he enquired about being important fatty acids (EFAs). Omega-6 and omega-3 fatty acids cannot be synthesised by the body, hence they must be obtained through food (Simopoulos 1999). Omega-6 fatty acids are pro-inflammatory in general. Omega-3 fatty acids reduce inflammation by inhibiting arachidonic acid's conversion to inflammatory prostaglandins and leukotrienes. The omega-3 fatty acids alpha-linolenic corrosive (ALA), eicosapentaenoic corrosive (EPA), and docosahexanoic corrosive (DHA) are the most common (DHA). The ratio of omega-6 to omega-3 fats in the human slim down has steadily increased from around 1-2:1 to over 25:1 since the

Paleolithic epoch. This surge appears to be linked to an increase in the number of chronic illnesses (Shinde *et al*., 1999) [11].

Gynandrophis pentaphylla, a member of the Capparaceae family, is one such plant that is predicted to regulate diabetes: provocative disorders. The mature plant grows to be around 1.3 metres tall, with compound (palmitate) takes off that have five leaflets. Its blooms are bisexual, with both male and female organs, earning it the name Gynandropsis (gynophore (female) and androphore (male) respectively (male). It has regular, hypogynous blooms. *Gynandrophis pentaphylla* is used to cure a variety of ailments, including headaches, epilepsy, stomach aches, ear discomfort, sepsis, diphtheria, vomiting, promoting labour toward the end of pregnancy, and snake bites. These homegrown drugs, which are linked to traditional healers and rural populations, should be tested for safety and efficacy, and feedback offered to the public. In this way, the experiment was carried out to determine the anti-diabetic and anti-inflammatory activity of *Gynandrophis pentaphylla in vitro,*

Materials and Methods

Plant materials

Leaves of *Gynandrophis pentaphylla* collected from the Government Siddha Medical College, herbal plant, Arumbakkam, Chennai, Tamilnadu, in September 2021, and taxonomically identified and authenticated as leaves by Dr. S. Sankaranarayanan, Head, and Office of Medicinal Botany. For future reference, a voucher specimen was deposited in the herbarium (Ref.No. MB/2021/Ceasal-387).

Phytochemical analysis

Standard methods were used to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins, and polyphenols in the aqueous leaf extract of *Gynandrophis pentaphylla* (Harborne 1973: Trease and Evans 1983).

Total phenolic content

The total phenolic content (TPC) of acetone extract from G. pentaphylla leaves was determined using Gutfinger's technique (1981). The methanol extract (1 mL, 1 mg/mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na2CO3 and centrifuged for 5 minutes at 13400Xg. After 30 minutes of incubation at room temperature, the absorbance of the upper phase was measured using a spectrophotometer (ELICO (SL150) UV-Vis Spectrophotometer) at 750 nm. As a catechol identical, the addition of phenolic material was transmitted.

Estimation of flavanoid

1 ml acetone extract from *G. pentaphylla* leaves was thoroughly mixed with 1 ml 2 percent aluminium chloride and 0.5 ml 33 percent acetic acid, followed by the expansion of 90 percent methanol, and the contents were completely mixed and allowed to stand for 30 minutes (Delcour and de Varebeke, 1985). A UV-Visible Spectrophotometer was used to detect the absorbance at 414 nm. As a benchmark, quercetin was used.

Thin layer chromatography profile

The acetone extract from the leaves of G. pentaphylla was stacked on to pre coated TLC (60 F2 54) and it was generated using a dissolvable system consisting of Petroleum ether, Chloroform, and methanol (1:0.5:0.1, V/V/V) was used to advance the exudates on silica gel plates silica gel 60 F254 (10x20 cm, 0.2mmlayer). It is luminous with UV light at 360nm and 240nm and is unmistakable and non-visible.

Glucose uptake in yeast cells

Rehashed centrifugation (3,000g, 5 min) of commercial baker's yeast in distilled water was performed until clear supernatant fluids were produced, and a 10% (v/v) suspension was prepared in distilled water. Plant extracts in various quantities (25-100 g/mL) were added to 1 mL of glucose solution (25 mM) and incubated for 10 minutes at 37 °C. The response was begun by adding 100 litres of yeast suspension, vortexing it, and then incubating it at 37 °C for 60 minutes. After 60 minutes, the tubes were centrifuged (2,500 g, 5 min) and the sum of glucose in the supernatant was determined (Cirillo, 1962). As a conventional medication, metronidazole was used. The following equation was used to compute the rate of increase in glucose uptake by yeast cells:

The absorbance of the control reaction is abs control (containing all reagents but the test sample) Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Inhibition of A-amylase activity

Alpha amylase is an enzyme that breaks down alpha bonds in expanding alpha linked polysaccharides like glycogen and starch to release glucose and maltose. The starch iodine method, which was first developed by (Hamdan and Fatimai 2010) and then used by others to ensure amylase activity in plant extracts with a few modifications, was used to test alpha amylase inhibitory activity. 1 ml potato starch (1 percent w/v), 1 ml acetone extract from G. pentaphylla leaves of various concentrations such as 25, 50, 75, and 100 g/ml, 1 ml alpha amylase enzyme (1 percent w/v), and 2 ml acetate buffer (0.1 M, 7.2 pH) were used in the alpha amylase inhibition method. NOTE: In an acetate buffer, potato starch, alpha amylase, and medication solutions were mixed together (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml refined water).Inhibition of alpha-

$$\text{Amylase (\%)} = \text{Abs sample} - \text{Abs control} \times 100$$

Inhibitory activity of A-glucosidase

The standard approach used by Dong *et al*. to assess -glucosidase inhibitory activity was used (2012). In 96 well plates, a volume of 60 l of test solution and 50 l of 0.1 M phosphate buffer (pH 6.8) containing -glucosidase solution (0.2 U/ml) were brooded for 20 minutes at 37 oC. After pre-incubation, each well was filled with 50 l of 5 mM p-nitrophenyl-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) and incubated for additional 20 minutes at 37 °C. At that time, the response was stopped by adding 160 l of 0.2 M NaCO3 to each well, and absorbance readings (A) were taken at 405 nm using a microplate peruser, and the results were compared to a control that had 60 l of buffer in place of the extract. Chemical arrangement was replaced by buffer arrangement and absorbance for clear incubation (to allow for absorbance created by the extract). The α-glucosidase inhibitory action was communicated as hindrance % and was calculated as takes after:

Anti-lipoxygenase activity

With modest adjustments, anti-lipoxygenase activity was investigated using linoleic acid as a substrate and lipoxidase as an enzyme (Shinde *et al*., 2012). Lipoxidase protein arrangement (20,000 U/ml) and 2M borate buffer pH 9.0 (0.25 ml) were used to dissolve test samples. At a temperature of 25°C, the reaction mixture was incubated for 5 minutes. Then 1.0 ml of 0.6 mM lenoleic corrosive arrangement was added. The reaction mixture was vortexed, and the absorbance at 234 nm was measured. As a control, diclofenac sodium was employed. The taking after equation was used to calculate the percent restraint.

% inhibition = Abs control -Abs sampleAbs control×100

Inhibition of albumin denaturation

The anti-inflammatory efficacy of acetone extract from *G. pentaphylla* leaves was investigated using Mizushima *et al*protocol.'s with a few modifications. The convention was followed when preventing albumin denaturation. An equal volume of test acetone extract from *G. pentaphylla* takes off of various concentrations (25-100 g/ml) and a 1 percent fluid solution of bovine albumin make up the reaction combination (Division V). A small amount of 1N HCl was used to balance the pH of the response mixture. The test extracts were heated to 51°C for 20 minutes after being incubated at 37°C for 20 minutes. After cooling the samples to room temperature, the absorbance was measured. Using an ultraviolet (UV)-visible spectrophotometer, the turbidity was measured at 660 nm. Protein denaturation rate restraint was estimated as follows:

% inhibition = Abs control -Abs sampleAbs control ×100

Result and Discussion

Phytochemical screening

The phytochemical screening of aqueous leaf extract from the leaves of *Gynandrophis pentaphylla* examined by and by appeared the presence of alkaloids, flavonoids, polyphenol, terpenoids, and nonappearance of glycosides and tannin (Table -1).

Table 1: Phytochemical screening of aqueous leaf extract from the leaves of *G. pentaphylla*

Sl. No.	Phytochemical Constituents	Observation	Aqueous leaf extract from the leaves of *G. pentaphylla*
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

According to the material and methods, the overall polyphenol content, antioxidant activity, and flavonoid content were determined using the folin-ciocalteau method, phosphomolybdenum assay, and aluminium chloride colorimetric approach, respectively.

In this situation, preliminary investigations revealed that acetone extract was the optimum solvent for extracting phenolics from G. pentaphylla leaves at 60 °C for 60 minutes since it yielded the most phenolics. The yields of acetone extract from *G. pentaphylla* leaves varied between 63.31 percent (w/w) and 53.32 percent (w/w).In this way, the entire phenolic and flavonoid component was broken down into catechin and rutin equivalents.

TLC profile

The acetone extract from the leaves of *G. pentaphylla* stacked on Pre-coated TLC plates (60 F2 54 Merck) and created with a solvent system of Toulene, dioxin and acidic acid within the proportion of 9.5:2.5:0.4. The developed plate was seen beneath UV 240nm and 360nm (Fig-1).

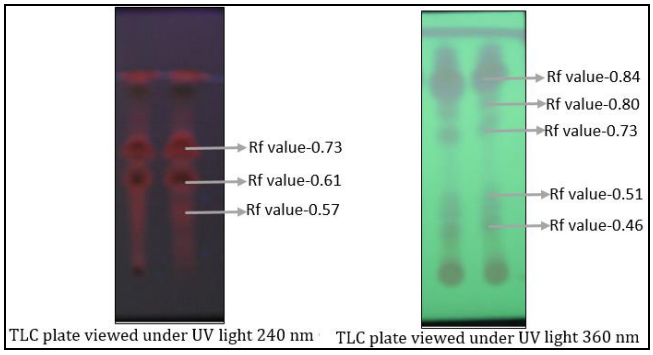
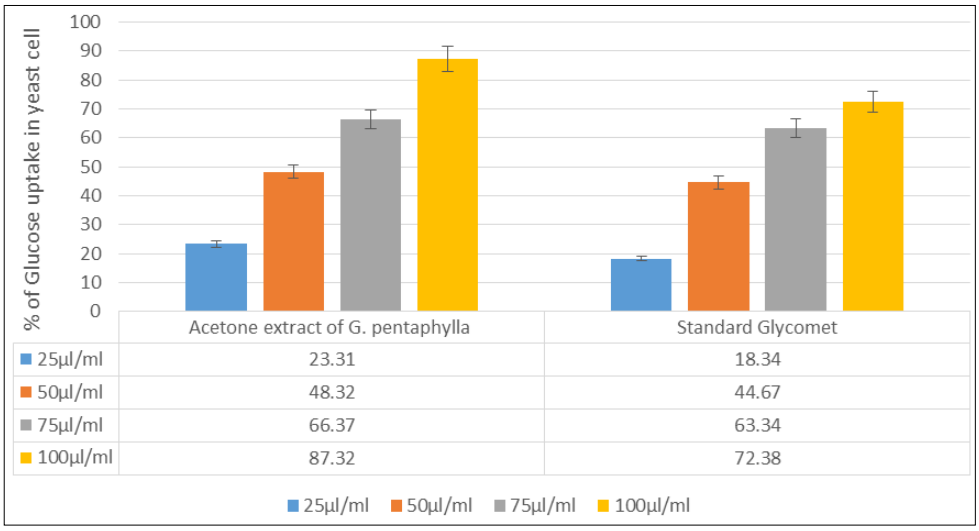


Fig 1: TLC profile of acetone extract from the leaves of *G. pentaphylla*

Glucose uptake in yeast cells of acetone extract from the leaves of *G. pentaphylla*

The rate of glucose transport over cell film in yeast cells framework is displayed in Graph-1. The amount of glucose remaining within the medium after a particular time serves as an indicator of the glucose take-up by the yeast cells. The rate of take-up of glucose into yeast cells

was straight in all the three glucose concentrations. The acetone extract from the leaves of G. pentaphylla shown significantly higher activity (87.32%) at all concentrations than standard (72.38%). In any case the most elevated take-up of glucose was seen in 25mM Glucose concentration. The result appeared the lower take-up of glucose by the yeast cells which conformed the most elevated action.

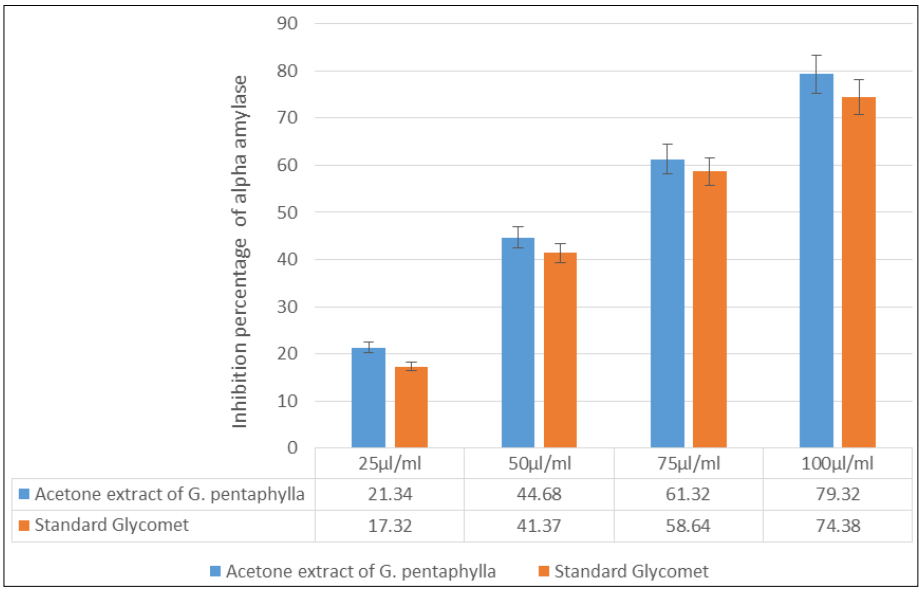


	Acetone extract of G. pentaphylla	Standard Glycomet
25µl/ml	23.31	18.34
50µl/ml	48.32	44.67
75µl/ml	66.37	63.34
100µl/ml	87.32	72.38

Graph 1: Glucose uptake in yeast cells of acetone extract from the leaves of *G. pentaphylla*

A-amylase inhibition of acetone extract from the leaves of *G. pentaphylla*

Alpha amylase is an enzyme that hydrolyses alpha-bonds of huge alpha linked polysaccharide such as glycogen and starch to abdicate glucose and maltose. Alpha amylase inhibitors tie to alpha-bond of polysaccharide and avoid break down of polysaccharide in to mono and disaccharide. In show test consider it was watched that acetone extract from the clears out of G. pentaphylla illustrated restraint of alpha amylase. But the result of acetone extract from the clears out of *G. pentaphylla* significant inhibition of alpha amylase activity (793.32%) as compared to standard drug glycomet (74.38%) (Graph 2) Different instruments, due to numerous phytoconstituents, were recorded for the antidiabetic action of restorative plants. Hence, archiving the viability of antidiabetic therapeutic plants has been expanded, and their characterizations of chemical constituents are centered in drug revelation programs (Tiwari and Rao, 2002) [12].



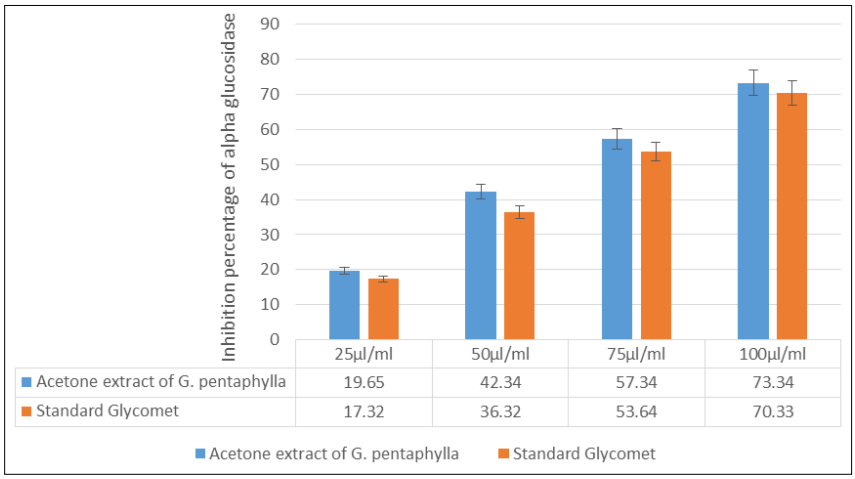
	25µl/ml	50µl/ml	75µl/ml	100µl/ml
Acetone extract of G. pentaphylla	21.34	44.68	61.32	79.32
Standard Glycomet	17.32	41.37	58.64	74.38

Graph 2: A-amylase inhibition of acetone extract from the leaves of *G. pentaphylla*

A-glucosidase inhibitory activity of acetone extract from the leaves of *G. pentaphylla*

The comes about of in-vitro α-glucosidase inhibitory study are appeared in Graph-3. The acetone extract from the takes off of G. pentaphylla appeared a concentration dependent inhibition of enzyme. The highest concentration of 100 µl/ml tested showed a maximum inhibition of about 73.34% acetone extract from the leaves of G. pentaphylla seems to be less potent in α-glucosidase inhibitory potential compared to glycomet (70.33%). It may be that α glucosidase is more delicate towards glycomet with the concentration required for 50% restraint (IC_{50}) found to be 66.38µg/ml. The key chemicals for carbohydrate metabolism within the little digestive system are pancreatic

α-amylase and α-glucosidase which change over expended polysaccharides to monosaccharides. This protein activity causes postprandial blood glucose level rise due to retention of formed glucose from polysaccharides within the small intestine. Unused drugs or definitions which are void of the over side impacts will make strides the compliance in type 2 diabetes



Graph 3: A-glucosidase inhibitory activity of acetone extract from the leaves of *G. pentaphylla*

Lipoxygenase inhibition activity of acetone extract from the leaves of *G. pentaphylla*

The inhibition of LOX utilizing linoleic acid as substrate was decided for the anti-inflammatory action within the acetone extract from the leaves of *G. pentaphylla*. The acetone extract from the leaves of *G. pentaphylla* at 100µl/ml concentration shown more hindrance than the other concentration. The inhibition rate was over 84.32% at 100µl/ml (Table-2). The standard diclofenac sodium was appeared 77.32% restraint at 100 µg/mL. The acetone extract from the leaves of *G. pentaphylla* was showed higher inhibition movement than positive control. Lipoxygenase catalyzes the expansion of atomic oxygen to greasy acids containing a cis, cis-1, 4 pentadiene framework. This response starts unsaturated greasy corrosive hydroperoxides. These items are advance changed over into others that play a key part in fiery forms. Subsequently, compounds which are able to hinder that protein can be considered as cancer prevention agents and having anti-inflammatory properties (Palmieri *et al*., 2011) [8].

Table 2: Inhibition activity of Lipoxygenase of acetone extract from the leaves of *G. pentaphylla*

Flavonoid Concentration	Acetone extract from the leaves of *G. pentaphylla*	Standard Diclofenac sodium
25 µl/ml	24.61±2.78	20.34±2.31
50 µl/ml	41.32±2.37	38.64±1.49
75 µl/ml	61.32±1.89	57.32±1.39
100 µl/ml	84.32±0.23	77.32±2.64
EC_{50} Vlaue	56.32±2.34	62.34±2.43

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 leaves of *G. pentaphylla*

Examination of acetone extract from the leaves of *G. pentaphylla* of earth shattering movement on inhibition of protein denaturation and its impact was compared with the standard drug Diclofenac sodium. The generation of auto antigen in certain arthritic disease may be due to denaturation of protein. From the comes about of show consider it can be expressed that alkaloid extract is capable of controlling the generation of auto antigen and restrains denaturation of protein in rheumatic illness. The greatest rate restraint of protein denaturation was watched in acetone extract from the leaves of G. *pentaphylla* 72.34% at 100 µg/ml which was near to the rate of inhibition of diclofenac sodium (68.32 %) (Table-3).

Table 3: Inhibition activity of protein denaturation of acetone extract from the leaves of *G. pentaphylla*

Different concentration	Acetone extract from the leaves of *G. pentaphylla*	Standard Diclofenac sodium
25 µl/ml	21.34±0.89	19.64±2.34
50 µl/ml	40.32±3.64	37.32±2.89
75 µl/ml	57.32±1.68	54.32±1.37
100 µl/ml	72.34±2.34	68.32±2.64
EC_{50} Vlaue	68.32±2.34	71.32±1.36

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

Conclusion

In vitro studies have shown that acetone extract from the leaves of *G. pentaphylla* has potential anti-diabetic and anti-inflammatory properties. The acetone extract from the leaves of *G. pentaphylla* was rationally bolstered in an *in vitro* investigation. Advance, acetone extract from *G. pentaphylla* leaves has active biomarkers that could potentially be responsible for antidiabetic action. The anti-inflammatory activity of acetone extract from the leaves of *G. pentaphylla* is likely due to enzyme inhibitors, according to this study.

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