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PHARMACOGNOSTIC STUDIES AND EVALUATION OF ANTI-DIABETIC EFFICACY OF THE LEAVES AND FLOWER OF *PHLOGACANTHUS THYRSIFLORUS*

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Abstract

Background of the Research: *Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both, with a global incidence of 422 million and 62 million in India. Phlogacanthus thyrsiflorus Nees is a medicinal plant used in treating diabetes, whooping cough, menorrhagia, wounds, tumours and as a blood purifier. It is also used for cold, cough, influenza, easy deliver of child birth, abortion, diarrhea, dysentery, cholera, high blood pressure control, boils, small pox, skin problems, sprains, body ache, constipation and burns.* ***Objective:*** *To determine pharmacognostic parameters for establishing qualitative and quantitative standards on Phlogacanthus thyrsiflorus leaf and flower, and to determine their possible antidiabetic property.* ***Methods:*** *Pharmacognostic standardization parameters were established following WHO guidelines and other Pharmacopoeial monograph. The antidiabetic study was done in-vivo in albino rats usingstreptozocin as the toxicant. The rats were treated for twenty-one days.* ***Results:****The leaf and flower contains alkaloid, carbohydrate, flavonoid and triterpenoid. Total ash, acid insoluble ash, water soluble extractive of the leaf and flower of 10.23± 0.30 and 7.99 ± 0.29, 1.96 ± 0.09 and 0.70 ± 0.23, 17.25 ± 0.33 and 16.05 ±0.35 respectively were recorded. From the data obtained, the leaf and flower produced a reduction in glucose level of the diabetic rats.* ***Conclusion:*** *The pharmacognostic data obtained may be used for proper identity and purity checking. The leaf and flower of Phlogacanthus thyrsiflorus produced significant antidiabetic effects in comparison to standard and diabetic control.*

Key Words: *Phlogacanthus thyrsiflorus* Nees, pharmacognosy, antidiabetic activity

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both (Ozougwu et al. 2013). With the help of the hormone insulin, cells throughout the body absorb glucose and use it for energy. Diabetes develops when the body doesn't make enough insulin or is not able to use insulin effectively, or both. The prevalence of diabetes is increasing day by day with the global rise of obesity and related life style diseases. Today, approximately 422 million adults are living with diabetes mellitus, majority (> 85%) of which is accounted for type-II diabetes (Junejo et al. 2017). Plant based medicine or herbal medicine play a vital role in treating various disorders including diabetes since time immemorial. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experience indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness" (Joshi et al 2015; Iris et al 2011; Verma et al 2008).

Phlogacanthus thyrsiflorus Nees, commonly known as '*Titaphul'* or *'Tita Bahokl*' in Assam is a well known medicinal plant belonging to the family Acanthaceae. Phyto- constituents such as β- sitosterol, stigmasterol, 8(17),13-labdadien-15,16-olide-19-oic acid, 19-hydroxy-8(17), 13-labdadien-15,16-olide were isolated from the dichloromethane extract and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone (Luteolin) were isolated from the 1-butanol part of methanol extract of flower. The leaves are reported to contain diterpene lactone, Phlogantholide A (Verma et al 2008; Chakravarty et al 2012; Ahmed et al 2016)

The plant can be seen growing mostly during December-April and is distributed throughout the tropics and in the entire North East Region of India. *Titaaphul* is found throughout subtropical Himalayas, from Garhwal to Bhutan, and North Bengal, at altitudes up to 1000m (Chakravarty et al 2012; Ahmed et al 2016; Dutta et al 2014).Whole plant is used in whooping cough and menorrhagia. Fruits and leaves are burnt and prescribed for fevers. In North Eastern India, flowers are used for treating wounds, tumours growth and as a blood purifier. Singh *et al.* reported the folk medicinal uses of *Phologacanthus thyrsiflorus* Nees,which includes treatment of cold, cough, influenza, easy deliver of child birth, abortion, irregular

menstruation, diarrhea, dysentery, cholera, high blood pressure control, boils, small pox, skin problems, sprains, body ache, constipation and burns (Singh et al., 2014). In Mishing community, *Phlogacanthus thyrsiflorus* leaves are used for treatment of loose motion. They extracted the leaf juice and taken orally with water (Shankar et al., 2012). In Assam, the leaves and flowers of this plant is used as one of the component in a herbal recipe comprising 101 vegetables (*Saak*) during 'BohagBihu', the main festival of Assam (Gogoi et al, 2015).

Various ethnobotanical studies reveal that the flowers as well as leaves of *Phlogacanthus thyrsiflorus* are very useful in curing diabetes. But, in depth scientific reports are missing to validate the traditional claims. Also, there is no report of detail pharmacognostic studies on this plant. Therefore, the present study was undertaken to study the scientific basis of using flowers and leaves of this particular plant for the management of diabetes mellitus using streptozotocin induced diabetes in experimental rats and to establish some pharmacognostic standardization parameters.

MATERIALS AND METHOD

Chemicals

Streptozotocin was purchased from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India. All other chemicals and reagents used were of analytical grade.

Collection of authentication Plant material

The leaves and flower of *Phlogacanthus thyrsiflorus*Nees were collected from Dibrugarh University Campus, Assam, in the month of July, 2016. The plant was identified and authenticated taxonomically by Dr. A.A. MAO, Scientist-F, at Botanical Survey of India, Shillong (No- BSI/ERC/Tech/2017/710 Dated: 27/3/2017).

Preparation of Plant extract

The dried leaves and flowers were pulverized in a mechanical grinder to coarse powder and then stored in air tight containers free from moisture. For preliminary phytochemical screening, powdered leaves and flowers were extracted with petroleum ether, chloroform, methanol and ethanol following cold maceration process for 48 hours with occasional stirring at a room temperature. Again, the powdered drugs (leaves and flower) were packed separately in a Soxhlet apparatus and

extracted with ethanol for 19 hours to yield ethanolic extract of *Phlogacanthus thyrsiflorus* (EEPT). The concentrated extract was dried in a rotary evaporator and stored in a desiccators for use in animal study experiments.

Study of pharmacognostic parameters

Micro and macroscopic evaluation

Microscopic assessment is an effective tool for determining identity of the plant material. Macroscopic evaluation is an assessment of the plant material, either with the naked eye or with simple magnification such as with a hand lens. Similar species of plants can share similar morphological characteristics and so appropriate training is needed to acquire macroscopic identification skill (Kokate et al 2012; Evans W.C. 2005; Mukherjee P. K. 2002)

Cytomorphological evaluation

Cytomorphology involves the examination of the cell and arrangement of different cells in a drug. A few amounts of finely powdered leaves and flower was boiled in a test tube with 5% KOH solution for 5 minutes and then taken in a glass slide. It was washed with water and then a pinch of powder was taken with glass slide, then a drop of clarifying agent was added along with one drop of glycerol. For the leaf part to remove the chlorophyll, the powdered drug was treated with sodium hypochlorite solution and hydrogen peroxide (6% v/v) and kept for overnight. The next day the tissues were washed with water to remove the chemicals and viewed under microscope after treating with different reagent viz. safranin/KOH (for identification of Ca Oxalate crystal), sudan red (for identification of suberized or cuticular cell wall), sudan red and ethanol (for identification of fats and fatty substances). The slides were then observed under Carl Zeiss trinocular microscope (Primostar) at two different magnifications (10x& 20x) and pictures were taken with True Charge IT Camera (Carl Zeiss) (Kokate et al 2012; Evans W.C. 2005; Mukherjee P. K. 2002).

Preparation of Section

The transverse sections of the leaves were obtained by cutting along the plane of a cylindrical portion of the leaf. A rectangular cut from the middle of the leaf was made as such that it contains the midrib. This was then embedded in the pith of potato pith so as to increase the surface area to make the sectioning process easy.

Free hand sections were obtained with the help of a sharp razor blade. The sections were heated mildly in a dilute solution of alkali (5% potassium hydroxide) to remove the chlorophyll. Then the section is stained with safranin and observed under microscope at different magnifications and pictures were taken with Primo Star ZEISS (Evans W.C. 2005; Khandelwal K. 2008).

Determination of Ash Value

The ash of any organic material is composed of their non-volatile inorganic components. More direct contamination, such as by sand or earth, is immediately detected by the ash value. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash and the water soluble ash. The total ash method designed to measure the total amount of material remaining after incineration. This includes both "Physiological ash", which is derived from the plant tissue itself, and "Non-Physiological ash", which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is that part of the total ash content which is soluble in water. It is the difference in weight between the total ash and the residue after treatment of the total ash with water (Kokate et al 2012; Evans W.C. 2005; Mukherjee P. K. 2002)

Determination of Extractive Values

The significance of determining the extractable matter refers to the amount of constituents present in a given amount of medicinal plant material extracted with solvents. Such extractable values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. Alcohol can dissolved almost all the substances, but in generally used for determining the extractive index for those drugs, which contain glycosides, alkaloids etc. Water is used for the drugs containing water-soluble substances as chief-constituents (Barreira et al 2008; Deore et al 2009)

Determination of loss on drying

The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis. Hence the moisture content of a drug should be determined and should

also be controlled. The moisture content of a drug needs to be minimized in order to prevent decomposition of crude drugs either due to chemical changes or by microbial contamination. Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. All the procedures were taken from the standard books and articles (Barreira et al 2008; Deore et al 2009)

Phytochemical investigation

Extraction

The method of extraction used in the study was Maceration as it results in lesser deterioration of the constituents. Maceration process is repeated successively with following solvents: Petroleum ether (40-60℃), Chloroform, Methanol, Ethanol. All the extracts obtained by extraction were subjected to various qualitative tests for the identification of various plant constituents present in the species.

Preliminary Phytochemical Tests

The different extracts were separately tested for the presence of various plant constituents such as alkaloids, carbohydrates, fats and oils, flavonoids, glycosides, proteins, steroids, triterpeniods, saponins, tannins and phenolic compounds (Kokate et al 2012; Evans W.C. 2005).

Fluorescence Study

Qualitative fluorescence analysis of powdered flowers and leaves was carried out using UV Chamber. A small quantity of dried and finely powdered sample was placed on a grease free microscopic slide and 1-2 drops of freshly prepared reagents were added by gentle tilting the slide and was waited for 1-2 minutes. The slides was placed inside the UV viewer chamber and viewed under daylight and long (366nm) ultraviolet radiations. The changes in colour were observed and recorded. The instrument that was used for detecting fluorescence was U.V.-Viewer Ultraviolet Fluorescence analysis cabinet, MAC® Macro Scientific Work (Kokate et al 2012; Chase et al 1949; Ansari et al 2009).

Thin Layer Chromatography (TLC)

Ascending thin layer chromatography was performed for the separation of phytoconstituents. Mobile phase selected for flavonoids were: Toluene: Ethyl acetate, Chloroform: Ethanol and Chloroform: Ethyl acetate. For Carbohydrate and Phenolic compounds Toluene: Ethyl acetate: Formic acid and n-Butanol: acetic acid: water was used.(Stahl 1969; Wagner et al 1996).

Anti-diabetic activity

Experimental Animals

Male albino rats of Wistar strain weighing about 80-200gm were obtained from Sh. Sanjay Saha, Proprietor, Saha Enterprise, Kolkata and used for experimental study. All the protocols were approved by the Instutional Animal Ethics Committee (IAEC), Dibrugarh University, Assam and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals); vide approval number 1576/GO/Ere/S/11/CPCSEA, dated: 30/3/2015.

Maintenance of Animals

The animals were housed in a well ventilated room, maintained at room temperature of 20-25 ± 2℃, 30-35% of relative humidity and 12 hours dark/light cycle. The animals were kept in large specious hygienic cages during the course of the experimental period and were provided with pellet diet and water ad libitum. Prior to the experimental study, animals were fasted by depriving them of food for 16 hr but allowing free access of water. Animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment, they were allowed to free access for water and standard rat feed.

Induction of Experimental Diabetes

Ethanolic extract of *Phlogacanthus thyrsiflorus* (EEPT) was assessed for antidiabetic activity in Streptozotocin (STZ) induced diabetic mice. Streptozotocin (65mg/kg,i.p.) treated rats were provided with 10% glucose solution after 3 hours for the next 24 hours to prevent fatal hypoglycemia.

Experimental Design

Animals with blood glucose level above 250 mg/dl were selected and divided in to five groups comprising five animals in each group. Group-I animals were considered as normal control which received distilled water only while, the group-II animals received a single dose of STZ (65 mg/kg, i.p.) and treated as diabetic control. Animals in group-III were diabetic and treated with standard drug, Metformin (10mg/kg, i.p.). Group-IV and group-V animals were also diabetic and received *P. thyrsiflorus* leaves extract and *P. thyrsiflorus* flower extract (200mg/kg each) respectively. Blood samples were collected from the tail vein of the overnight (12-15 hr) fasted rats and blood glucose level was determined on 0th, 5th, 10^{th}, 15th and 21^{st}day. On the 21^{st}day all the animals were sacrificed and blood were collected from the animals and centrifuged at 4000 rpm for 5minutes to separate the serum for estimation of biochemical parameters. Histopathological study of the pancreas tissue was also done using a rotary microtome (ICON Instruments) and eosin-haematoxylin staining (Selvan et al., 2008; Gandhi, 2012).

Biochemical investigation

At the end of 21^{st}day of treatment blood samples were collected from the animals and centrifuged at 4000 rpm for 5minutes to separate the serum. From the serum different biochemical parameters like Total cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG), Aspartate transaminase (SGOT), Alanine transaminase (SGPT), Alkaline phosphatase (ALP) and Total Protein levels (TP) in serum were measured colorimetrically (Heerspik 1980)

Statistical analysis

All results were expressed as mean ± SEM. The significance of the difference between the means of test and control studies was established by student's t-test. P value less than 0.01 and 0.05 were considered significant.

Results

In macroscopical studies, the colourof fresh leaf was found dark green (upper surface) and light green (lower surface) while the dried powder colour was found yellowish green. Size of the leaf was 20×2, odor was characteristic, taste was bitter and surface was smooth and hairless. In case of flower, it was found that its colour

in fresh condition was orange and in dried flower was reddish brown. Size of the flower was wide tube shaped with two lipped, odor was characteristic, taste was bitter and surface was smooth and hairy.

In the powder microscopy of leaves (10x) showed stomata, fiber, starch, vessel, Ca Oxalate crystal and cuticular cell wall while the presence of trichomes and pollen are noticed in flower. Transverse sections of leaf showed prominent single layer of upper and lower epidermis. Cuticle cells are present below the upper epidermis and Palisade parenchyma present just below the cuticle as shown in Fig. 2 and 3. Xylem and phloem are also observed which were surrounded by the prominent endodermis layer. Few collenchyma cells are present above the lower epidermis.

Physicochemical parameter like total ash of leaves (10.23±0.30) and flowers (7.99±0.28), acid insoluble ash of leaves (1.96±0.08) and flowers (0.70±0.23), water soluble ash of leaves (8.03±0.29) and flowers (7.37±0.59) were recorded. Water soluble extractive value of leaves (17.25±0.33) and flowers (16.05±0.35), alcohol soluble extractive value of leaves (23.75±0.46) and flowers (20.50±0.49) and also loss on drying values of leaves(15.39±0.40) and flowers (13.88±0.40) were estimated. The percentage yield of extraction was found to be 0.85%, 1.95%, 1.55% and 1.65% (w/w) respectively for petroleum ether, chloroform, methanol and ethanol extracts. Preliminary phytochemical tests revealed that chloroform extract of *Phlogacanthus thyrsiflorus* possesses highest amount of phytoconstituents as compared to that of other three extracts i.e. petroleum ether, methanol, and ethanol extracts (Table 2).

The powdered drug exhibit different fluorescence characters (Table 3) which might be due to the presence of different functional groups in drug chemical constituents. The chemicals such as H_2SO_4, HCl, HNO_3, NH_3 etc., in different proportions may change the configuration of the functional groups present in the powdered drug and changes in the color occur.

TLC fingerprinting was performed with the aim of characterizing and identifying the individual components in the different solvent extract. The R_f values indicates the position at which a substance is located in a chromatogram (Table 4 and 5).

The effect of Test drugs (200mg/kg of bodyweight) i.e. ethanolic extract of *Phlogacanthus thyrsiflorus*(EEPT) leaves and flower on fasting blood glucose levels were investigated in the Streptozotocin induced diabetic Wister albino rat

using metformin hydrochloride as standard drug (10 mg/kg of bodyweight). The divergence of body weights of the animals and the mean blood glucose levels of controlled groups and test groups were noted as shown in the Table 6 &7 and in Fig. 4 on 0th, 5th, 10^{th}, 15^{th} and 21^{st} day of treatment. Hypoglycemic effect was observed in animals treated with ethanolic leaves and flower extract of *Phlogacanthus thyrsiflorus* Nees was noted. SGPT, SGOT, ALP, total cholesterol and triglyceride were significantly higher in STZ treated mice compared to the normal mice except total protein value which was lower in diabetic control. The continuous treatment with the leaves and flowers extract of *Phlogacanthus thyrsiflorus* for 21 days brought the lipid parameters in the diabetic mice to almost normal levels as shown in Table 8.The EEPT leaves showed better result compared to the EEPT of flower. Treatment with leaf EEPT could able reverse the increased levels of these lipid ratios and normalized soon after.

Histopathology

The histopathological studies of pancreas (Fig. 5) of STZ-treated diabetic rats exhibited reduction in dimension of islets, damaged beta cells and extensive necrotic changes followed by fibrosis and atrophy (5B). Both standard (metformin) and test (EEPT leaves & flower 200 mg/ kg) drug effectively restored the necrotic and fibrotic changes and also increase the number and size of the islets (5 C, D & E). The pancreatic cells in vehicle treated group showed normal acini and normal cellular islets of langerhans (5A). There was no loss of cellular integrity and no prominent necrosis and fatty degeneration observed. The changes in pancreas morphology in metformin treated group are nearly similar to test drug treated rats where changes are significant ($p< 0.05$) compared to vehicle control group.

Discussion

Search for newer and safe alternative is the work horse behind the discoveries of bioactive compounds from natural sources with improved features including the inseparable safety and efficacy criteria. There have instances in the history when the scientific evaluation of traditionally popular plant material yielded moieties that became the classical back bone for a particular pharmacological class of drugs. While on the far end it had led to the discovery of cloaked potential of the plant which has never been expected and nor its use was traditionally related to that of the prevalent one. Hence the scientific measures of authentication of a plant for a

particular disease important in the aspect mentioned above, although the result obtained in such study must be properly scrutinized and correlated with the phytoconstituents present.

The plant *Phlogacanthus thyrsiflorus*Nees.is an important and endemic plant of North East India. As literature suggests this plant is distributed throughout the tropics and in the entire North East region of India. This plant has been used by many ethnic communities of Assam to treat various disease ailments including diabetes. It is very commonly used as a folk medicine in Assam.

Based on this, a systematic study was carried out with the plant in order to explore the pharmacognostic, preliminary phytochemical and pharmacological activities with special reference to its antidiabetic potential. During the course of this project work, an attempt was made to study all the pharmacognostic parameters of the plant so as to facilitate proper identification of the plant.

The pharmacological parameters are evaluated in order to authenticate the potential of the plant to lower the blood glucose has been believed. The pharmacognostic and phytochemical studies will help for correct identification of this plant for future reference. The preliminary phytochemical studies exhibited the presence a numbers of phytoconstituents of medicinal importance such as alkaloids, terpenoids and flavonoids in the different extracts of leaves and flower of *Phlogacanthus thyrsiflorus*.

In-vivo evaluation of the antidiabetic activity of ethanolic extract of the leaves and flower of *Phlogacanthus thyrsiflorus* (EEPT) was carried out for 21 days animal study protocol and measurement of different biochemical parameters, and Histopathological study against Streptozotocin induced diabetes in experimental rat. From the histopathological studies it was evident that in normal control group the islet boundaries were clear and the profiles of the islet cells were clearly visible. No necrosis or fatty degeneration was observed. The pancreatic cellscontain well preserved cytoplasm and nucleus. After inducing STZ, in diabetic control group, extensive destruction of cells was observed with ballooning and necrosis. Normal cellular integrity was completely lost along with fatty degeneration indicating a clear sign of toxicity. However, in EEPT of leaves (200 mg/kg b.w.) the overall architecture of the cells seemed to be reverted back to normal and the integrity of the cells were also very much better as compared with the EEPT of flower (200

mg/kg b.w.). In the standard drug (metformin 10 mg/kg b.w.) treated group, the islets cells were observed as normal in position. But number of islet cells were seems to be less as compared to the normal group. The architecture of acinar cells and size were back to normal upon the standard drug treatment. Histopathological studies of pancreas in diabetic and treated groups substantiate the cytoprotective action of extract.

Table1: Ash values, Extractive values & Loss on drying expressed as Mean ± SEM

Sl. No.	**Parameters**	**Mean ± SEM**	
		Leaves	**Flower**
1.	Total ash	10.23± 0.30	7.99 ± 0.28
2.	Acid insoluble ash	1.96 ± 0.08	0.70 ± 0.23
3.	Water soluble ash	8.03 ± 0.29	7.37 ± 0.59
4.	Water soluble extractive	17.25 ± 0.33	16.05 ± .35
5.	Alcohol soluble extractive	23.75 ± 0.46	20.50 ± 0.49
6.	Loss on drying	15.39 ± 0.40	13.88 ± 0.40

Table 2: Preliminary phytochemical test of leaf and flower extracts of *Phlogacanthus thyrsiflorus*Nees.

Plant constituents	**Petroleum ether**		**Chloroform**		**Methanol**		**Ethanol**	
	Leaf	**Flower**	**Leaf**	**Flower**	**Leaf**	**Flower**	**Leaf**	**Flower**
1. Alkaloids	-	-	+	+	+	-	-	-
2. Carbohydrate	-	-	-	-	+	+	+	+
3. Fats and fixed oil	+	+	-	-	-	-	-	-
4. Flavonoids	-	-	+	+	+	+	-	-
5. Glycosides	-	-	-	-	-	-	-	-
6. Triterpenoids	-	-	+	-	+	+	+	+
7. Tannin	-	-	-	-	-	-	-	-
8. Proteins	-	-	-	-	-	-	-	-
9. Saponins	-	-	-	-	-	-	-	-

Table 3: Fluorescence Study of powdered leaves of *Phlogacanthus thyrsiflorus* Nees.

Sl. No.	Particulars of the treatment powder +(reagent)	Under day light	Under UV light 366 nm
1.	No reagent	Dark Green	Yellowish green
2.	Conc. H_2SO_4	Reddish Brown	Black
3.	Distilled water	Dark green	Greenish
4.	Liquid NH_4	Greenish	Greenish yellow
5.	Conc. Nitric acid	Dark green	Light brown
6.	Iodine solution (1%)	Dark green	Brownish green
7.	NaOH solution(1N)	Reddish yellow	Pale yellow
8.	Dil. HNO_3	Brownish	Blackish
9.	Conc.HNO_3+NH_3	Dark green	Bluish green
10.	Conc. HCl	Pale brown	Dark brown

Table 4: TLC of various leaves extract of *Phlogacanthus thyrsiflorus* Nees.

Extract	Solvent system	Ratio of solvent used	No. of spot	R_f Values	Visualizing agent
Petroleum ether	Toluene:Ethyl acetate	9:1	1	0.77	1% Vanilla in conc. H_2SO_4
		8:2	1	0.87	
	Toluene:Ethyl acetate: Formic acid	9:1:1	1	0.45	
		8:2:1	1	0.85	
Chloroform	Toluene:Ethyl acetate	9:1	2	0.77, 0.31	1% Vanilla in conc. H_2SO_4
		8:2	1	0.55	
	Toluene:Ethyl acetate: Formic acid	9:1:1	2	0.22, 0.86	
		8:2:1	2	0.45, 0.89	

(Contd.)

(Contd.)

Methanol	Chloroform: Ethanol	3:1	3	0.21, 0.38, 0.83	Iodine Chamber
	Chloroform:Ethyl acetate	3:1	3	0.9, 0.54, 0.37	
		2:2	2	0.91, 0.51	
	Toluene:Ethyl acetate	2:8	3	0.66, 0.84, 0.62	
		1:9	3	0.84, 0.54, 0.22	
Ethanol	Chloroform: ethanol	3:1	2	0.46, 0.85	Iodine Chamber
		2:2	2	0.36,0.83	
	Chloroform:ethyl acetate	3:1	3	0.89, 0.55, 0.82	
		2:2	2	0.91, 0.51	
	Toluene:ethyl acetate	2:8	2	0.86, 0.66	
		1:9	2	0.55, 0.83	

Table 5: TLC of various flowers extracts of *Phlogacanthus thyrsiflorus* Nees.

Extract	Solvent System	Ratio of Solvent used	No. of spot	R_fValues	Visualizing agent
Petroleum ether	Toluene:ethyl acetate	9:1	1	0.79	1% Vanilla in conc. H_2SO_4
		8:2	2	0.69, 0.89	
	Toluene:ethyl acetate: formic acid	9:1:1	1	0.86	
Chloroform	Toluene:ethyl acetate	9:1	2	0.73, 0.88	1% Vanilla in conc. H_2SO_4
		8:2	2	0.86, 0.92	
	Toluene:ethyl acetate: formic acid	8:2:1	1	0.57	
Ethanol	Chloroform: ethanol	3:1	2	0.76, 0.95	Iodine Chamber
		2:2	1	0.86	
	Chloroform:ethyl acetate	3:1	2	0.54, 0.307	
		2:2	2	0.34, 0.69	
	Toluene:ethyl acetate	9:1	2	0.54, 0.92	
		2:8	1	0.57	
	n-Butanol:acetic acid: distilled water	4:1:1	1	0.8	
Methanol	Chloroform: ethanol	2:2	1	0.70	Iodine Chamber
	Toluene:ethyl acetate	1:9	1	0.70	
		2:8	1	0.71	
	n-Butanol:acetic acid: distilled water	5:0.5:0.5	1	0.90	
		4:1:1	1	0.83	

(Contd.)

Table 6:- The deviation of body weight of the animals during the treatment with EEPT leaves and flower

Animal group	**0th day**	**5th day**	**10th day**	**15th day**	**21th day**
G-I Normal control	92.86±1.94*	93.22± 3.65*	92.8 ± 2.63*	92.4± 2.74*	93.88± 2.09*
G-II Diabetic control	116.4±1.69*	105.4± 7.62*	104 ± 5.28*	102.6± 6.18*	91.74± 2.70*
G-III Standard treated)	90.4± 3.41**	87.6 ± 6.94*	91.0± 6.15*	91.8± 8.84*	92.5± 8.37*
G-IV EEPT Leaves (200 mg/kg BW)	106.67± 4.24*	99.0 ± 5.78*	103.6± 6.51*	105.4 ± 3.34*	105.42± 5.82*
G-V EEPT Flower (200 mg/kg BW)	99.2± 1.62**	92.4 ± 2.24*	94.2 ± 3.72*	92.6± 4.44*	93.38± 3.61*

Values are expressed as mean± SEM (n=5), statistical significance:**P<0.01, *P>0.05, compared with that of diabetic control group II.

Table 7: The effect of EEPT leaves and floweron fasting blood glucose level on Streptozotocininduced diabetic rats.

Treatment	0th day	5th day	10th day	15th day	21th day
G-I Normal control	92.86± 2.21	92.40 ± 3.41	88.00 ± 4.46	96.20± 3.08#	97.80 ± 3.31
G-II Diabetic control	115.40±3.75**	145.60±11.66#	220.80±59.07*	233.40± 83.37#	301.40± 88.54**
G-III Standard treated	105.80± 5.53#	183.80± 28.97**	162.80± 24.28#	120.40± 10.88#	111.60± 7.35#
G-IV EEPT Leaves (200 mg/kg BW)	114.2±5.66*	146.40 ± 8.75#	121.00 ± 9.53#	111.60± 7.68#	116.00± 7.06#
G-V EEPT Flower (200 mg/kg BW)	108.40± 6.38#	168.20± 17.64*	145.00± 16.914#	133.00± 4.29#	129.20± 5.54#

Values are mean ± SEM (n=5); statistical significance: ** P<0.01, #P>0.05, *P<0.05; compared with Normal control group I.

Table 8:- Serum Biochemical parameters of treated and control groups

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
G-I Normal control	29.25± 3.32$^{\#}$	20.00± 0.40**	11.50± 0.64**	7.45± 0.26**	69.05± 0.56**	72.12± 0.42**
G-II Diabetic control	40.75± 2.95	48.25±3. 11	19.00± 1.87	5.25± 0.47	93.50± 1.19	196.95± 2.82
G-III Standar d control	21.75± 3.19*	21.00± 1.29**	11.25± 0.62**	6.45± 0.23*	79.50± 0.64**	74.25± 1.31**
G-IV EEPT Leaves (200mg/ kg)	25.25± 1.70**	23.75± 2.52**	12.00± 0.91**	7.10± 0.14**	82.00± 1.35**	76.25± 1.31**
G-V EEPT Flower (200mg/ kg)	31.25± 3.49$^{\#}$	26.25± 1.75**	16.00± 0.70$^{\#}$	6.20± 0.20$^{\#}$	84.00±0.40 **	78.75±0.47* *

Values are mean ± SEM (n=5), statistical significance:$^{\#}$ P > 0.05, **P < 0.01, *P<0.05; compared with diabetic control group II.

Fig.1: Leaf and flower of *Phlogacanthus thyrsiflorus* Nees (Acanthaceae)

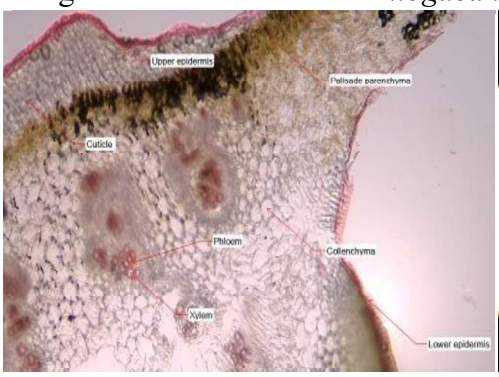


Fig.2: T.S. of Leaves (40x)

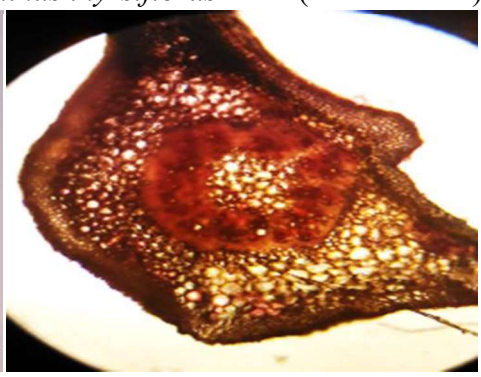


Fig.3: T.S. of Leaves (10x)

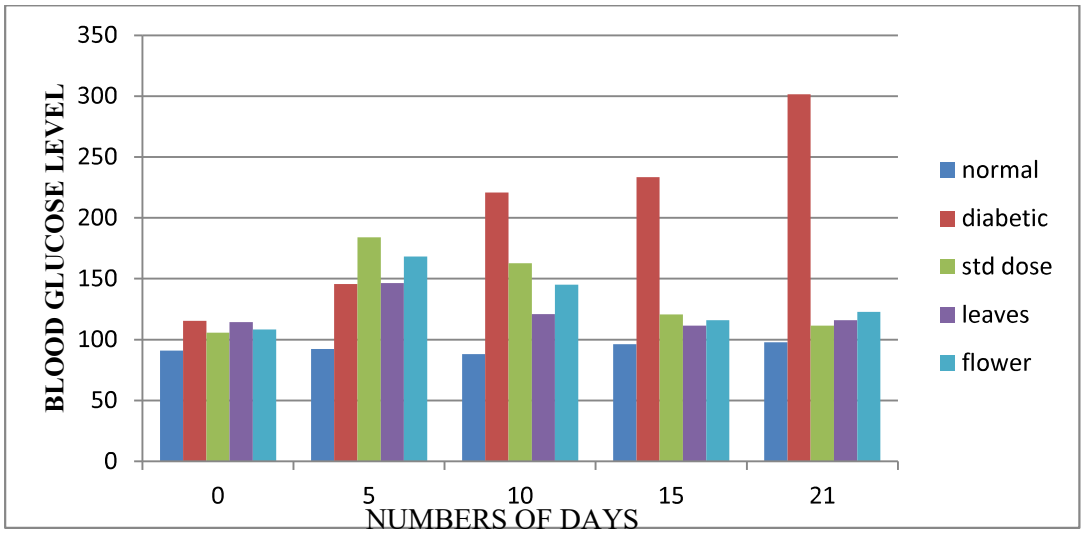
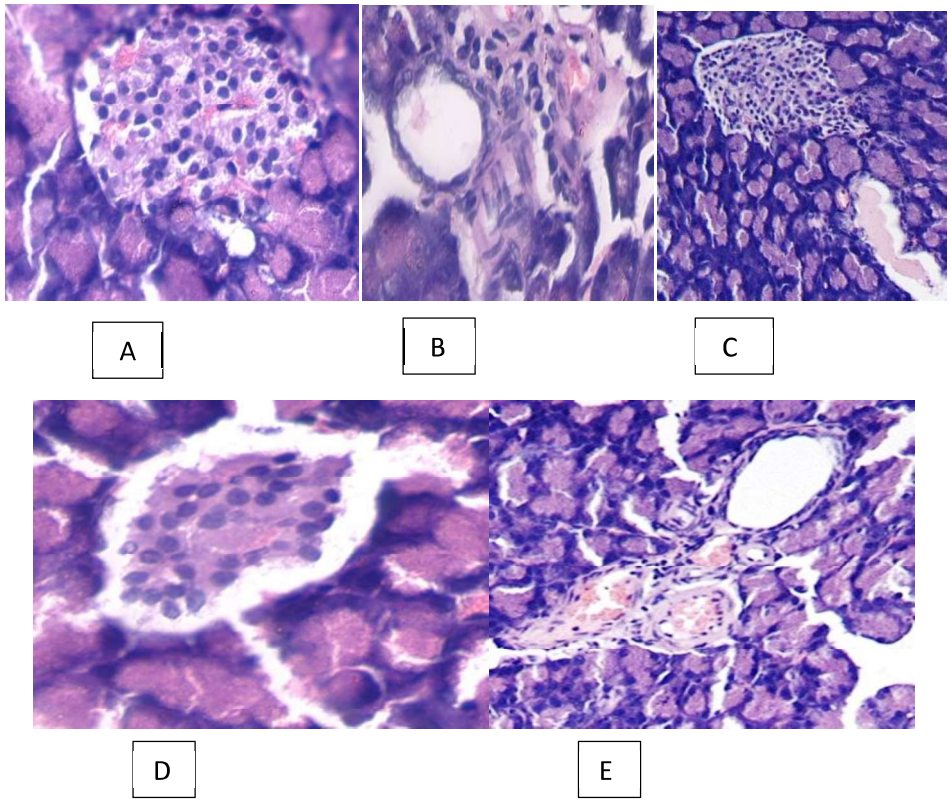


Fig. 4: Fasting blood glucose level



· **Fig 5:** Photomicrograph of pancreatic cells in normal (A), diabetic control (B), Std. drug treated (C), EEPT leaves treated (D) and EEPT flower treated (E) rats.

Conclusion

In the light of result, our study revealed that *Phlogacanthus thyrsiflorus* is an important traditional medicinal plant of North East India with promising antidiabetic potential. However, the detailed study is required in terms of its isolated compound and their activities. Further studies will be required to know the possible mechanism of action particularly in terms of reducing the increased blood glucose levels.

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