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Chemistry and Antidiabetic Effects of *Phlogacanthus thyrsiflorus* Nees Flowers

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Abstract

The present study was carried out to evaluate the chemical constituents and antidiabetic activity of flower extracts of *Phlogacanthus thyrsiflorus* Nees in streptozotocin induced diabetic Long-Evans rats by feeding for 28 days. The ethanolic extract (1.25 mg/kg bw) exhibited significant reduction of fasting serum glucose level in diabetic rats. The ethanolic extract also showed improvement in parameters of lipid profile. Five compounds namely, β- sitosterol (1), stigmasterol (2), 8(17),13-labdadien-15,16-olide-19-oic acid (3), 19-hydroxy-8(17),13-labdadien-15,16-olide (4) were isolated from the dichloromethane extract and 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone (Luteolin) (5) were isolated from the 1-butanol part of methanol extract of flowers. The structures of these compounds were elucidated by extensive spectroscopic studies. This is the first report of isolation of compounds (1-5) from flowers of this plant. The activities of the flower extract may be due to the presence of some of these compounds which demands further studies.

Keywords: *Phlogacanthus thyrsiflorus* Nees; Streptozotocin; Type 2 diabetic model rats; Hypoglycemic effect; Isolation of compounds

Introduction

Medicinal plants from time immemorial have been used frequently for the treatment of diabetes [1,2]. Dependence on plant materials for managing diabetes mellitus is increasing due to its easy availability and low cost with often wrongly perceived reduced toxicity [3,4]. Therefore, with up surging interests in antidiabetic plant materials, there is an urgent need for thorough scientific investigations of many potential plants for both efficacy and potential toxicities. However, the challenge of determining specific active components with good pharmacological activities still remains [5].

Phlogacanthus thyrsiflorus Nees (Acanthaceae) locally known as rambasak is a large shrub found usually in the sub-tropical Himalayas, Bihar, North Bengal, Assam and Bangladesh [6]. The shrub grows during May to August. The leaves are elliptical. The flowers are yellow in colour. Flowers taste bitter when it is taken after frying. It grows in forest and regenerated from seeds [7] and flowering occurs in the month of February to April [8]. Different parts of this plant are being used for the cure of different ailments like, fever, gastritis, pharyngitis, cough, bronchial asthma, rheumatism and many more [9]. This botanical herbaceous plant is widely used as verdant foods, medicine practice and traditional medicines. The aqueous extract of the flower of *P. thyrsiflorus* have been claimed to possess significant reduction of blood glucose level ($p<0.0001$), serum cholesterol ($P<0.01$) and increase in liver glycogen ($P<0.0001$) on steptozotocin induced diabetic mice [10]. The leaves are reported to contain diterpene lactone, phlogantholide, saponins and flavonoids and their glycosides [9]. The objective of this work was to investigate the scientific basis for its use in the treatment of diabetes mellitus. Therefore, the study was designed to evaluate the antidiabetic effect of *P. thyrsiflorus* flowers on type 2 diabetic model rats and isolate compounds from it.

Experimental

General

^{1}H and ^{13}C-NMR spectra including DEPT, 1H-1H COSY and HMBC spectra were recorded on BRUKER DBX-400 MHz NMR and VARIAN 500 MHz NMR spectrometers. The chemical shifts are reported in ppm with respect to residual non-deuterated solvent signals. The mass spectra were recorded at 70 ev with Finnigan 4021 and GCMS- QP2010 Ultra instrument. The fast atom bombardment mass spectrum ($FAB^{+}MS$) was recorded as a positive ion mode with m/z ranging between 0.0020-1000.0000.

Plant material

Flowers of *Phlogacanthus thyrsiflorus* Nees were collected from Dibrughar, Assam, India. The flower was identified by Taxonomist of Bangladesh National Herbarium (Accession number 42992). The collected materials were cleaned, air dried and finally dried in an oven at 40°C. The dried flowers were ground to a course powder with a cyclotec grinder.

Extraction

The powdered flowers (300 g) of *Phlogacanthus thyrsiflorus* Nees were successively extracted at room temperature with dichloromethane

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(DCM) (2 L × 4: 24 hrs) followed by ethyl acetate (2 L × 4: 24 hrs) and methanol (2 L × 4: 24 hrs). The extracts were filtered off and the filtrates were evaporated to dryness at 40°C with rotary vacuum evaporator and finally freeze dried to afford 8.1 g of DCM, 3.0 g of ethyl acetate and 16 g of methanol extracts. The powdered flowers (800 g) were also successively extracted with aq 80% ethanol (2 L × 6 times) to give 14 g of aq 80% ethanol extract. The residue (142 g) was suspended in water (3 L) and partitioned with DCM (3 L × 3 times) followed by 1-butanol (3 L × 3 times). The DCM and 1-butanol soluble parts were evaporated to dryness to get DCM (36.59 g) and 1-butanol (54.0 g) soluble extractives.

Isolation of compounds

The first dichloromethane extract (8.1 g) was fractionated by column chromatography (silica gel) using gradient elution of n-hexane, ethyl acetate and methanol with 10% increment and fourteen fractions ($1F_1$-$1F_{14}$) were collected. Fraction $1F_5$ was again fractionated over a silica gel column and eluted gradually with n-hexane and the polarity was increased by adding ethyl acetate and methanol in different proportion and six fractions ($2F_1$-$2F_6$) were collected. Compound **1** was purified as needle shaped crystal from fraction $2F_5$ by washing the crystal with n-hexane and methanol. Fraction $2F_6$ was purified to give compound **2** over a silica gel column and eluted with the same mobile phase. Compound **2**, (R_f value of 0.54) was isolated as white needles, after washing the crystal with dichloromethane and methanol.

Fraction $1F_{11}$ showed brownish crystals. Then the fraction was again fractionated over a silica gel column and eluted gradually with n-hexane and the polarity was increased by adding ethyl acetate and methanol in different proportion and fourteen fractions ($3F_1$-$3F_{14}$) were collected. Fraction $3F_5$ showed crystal and was purified to Compound **3** by washing the crystal with the same solvent n-hexane and methanol. Fraction $3F_8$ also showed crystal. Compound **4** was isolated by washing the crystal with the same washing solvents.

The butanol extract (52.0 g) was chromatographed over Sephadex LH-20 column and eluted gradually with deionized water and methanol mixtures with decreasing polarity and fractions ($4F_1$-$4F_{71}$) were collected. Fraction $4F_{35}$ was purified to compound **5** (19.0 mg) by HPLC [Column RP-18 (250 mm × 4.6 mm i.d.), mobile phase 60% aqueous acetonitrile, flow rate: 1.0 mL/min, UV detection at 254 nm and column oven temperature 37°C]. The isolated compounds had the following characteristics:

Compound **1** was needle shaped crystals with m.p. 137-38°C. The ^{1}H- and ^{13}C-NMR spectral data ($CDCl_3$) are presented in Tables 1 and 2, respectively.

Compound **2** was needle shaped crystals with m.p. 162.5°C. The crystal of the compound was soluble in $CHCl_3$. From the ^{1}H- and ^{13}C-NMR spectral data ($CDCl_3$) are presented in Tables 1 and 2, respectively.

Compound **3** was long fatty shaped crystals. It gave single spot on TLC with R_f value 0.53 (n-hexane : ethyl acetate; 3:7). The ^{1}H- and ^{13}C-NMR spectral data ($CDCl_3$) are presented in Tables 1 and 2, respectively. GCMS m/z 332 [M+H$^+$]. FTIR (KBr) ν_{max}: 2940, 1691, 1528, 669 cm^{-1}.

Compound **4** was white powder. It gave single spot on TLC with R_f value 0.50 (n-hexane : ethyl acetate; 4:6). The ^{1}H- and ^{13}C-NMR spectral data ($CDCl_3$) are presented in Tables 1 and 2, respectively. GCMS m/z 334 [M+H$^+$]. FTIR (KBr) ν_{max}: 2935, 1714, 1528, 670 cm^{-1}.

Compound **5** was brownish powder. The molecular mass of this compound was found to be 286 g/mol. From the ^{1}H- and ^{13}C-NMR spectral data ($CDCl_3$) are presented in Tables 1 and 2, respectively.

Experimental animals

The study was conducted on adult Long-Evans rats of both sexes (weighing 180-220 g). They were bred at the BIRDEM animal house and maintained at a constant room temperature of 22°C**,** 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food and water except the day of experimental procedure when animals were used after 12 hrs fasting. The rats had no access to food during the whole period of blood sampling. The influence of circadian rhythms was avoided by starting all the experiments at 8.30 a.m. Experiments on the animals were performed following the guidelines approved by Bangladesh Association for Laboratory Animal Science.

Induction of Type 2 diabetes to the rats

Type 2 diabetes was induced by a single *intraperitoneal* injection of streptozotocin (STZ, Upjohn Company, Kalamazoo, MI, USA) at a dose of 90 mg/kg body weight to 48 hrs old pups.

Three months later of STZ injection an oral glucose (2.5 g/kg bw) tolerance test was performed and type 2 diabetic model rats (blood glucose level 7-9 mmol/L at fasting condition) were selected to carry out the experiment with ethanolic extract of *P thyrsiflorus* flowers. The length of experiment was 28 days. A total of 18 Type 2 rats were used in this experiment. The rats were divided into the following three groups of six rats in each group:

1) Type 2 control group (fed with water)

2) Type 2 positive control group (fed with glibenclamide at a dose of 5 mg/kg bw)

3) Type 2 treated group (fed with 80% ethanol extract of *P. thyrsiflorus* at a dose of 1.25 gm/kg bw/10mL of water).

The rats were fed consecutively for 28 days with a single feeding every day. The body weight of each rat was measured on day 0, 7, 14, 21 and 28 day of experimental period.

Collection of blood samples for biochemical procedures

The rats were kept fasting for 12 hrs on the 0 day and blood was collected from the fasted rats by amputation of the tail tip under (diethyl ether) anesthesia. On the 29th day after 12 hrs fasting blood was collected from the rats by cardiac puncture. The collected blood samples were centrifuged; the serum was separated and kept frozen at -20°C until analysis of different biochemical parameters.

Biochemical analysis

The parameters measured were: serum glucose (glucose-oxidase), serum insulin (ELISA) and serum lipids by enzymatic-colorimetric method.

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD as appropriate. Statistical analyses of the results were performed by using the student's t-test or ANOVA (analysis of variance) followed by Bonferroni post hoc test. The limit of significance was set at $p<0.05$. In addition, the percentage changes compared to the initial value of the corresponding groups of rats were calculated to help the understanding of the effect of *P. thyrsiflorus* flowers on different parameters studied.

Position	δ_H, Multi (*J* in Hz)				
	1	2	3	4	5
1	-	-	1.78 (m)	1.78 (m)	-
2	-	-	1.63 (m)	1.65 (m)	-
3	3.52 (1H, m)	3.52 (1H, m)	1.67 (m)	1.69 (m)	(6.53, s)
4	-	-	-	-	-
5	-	-	1.97 (d; J=11.65 Hz)	1.96 (d; J=11.65 Hz)	-
6	5.35 (1H, brs)	5.35 (1H, brs)	1.53 (dddd/dq, J=4.15, 12.90, 25.65 Hz) 1.41 (d; J=10.75 Hz)	1.53 (dddd/dq, J=4.15, 12.90, 25.65 Hz) 1.40 (d?; J=10.75 Hz)	(6.43, br s)
7	-	-	2.40 (d; J=12.75 Hz) 2.08 (ddd/dt; J=4.12, 12.35, 16.80, 24.7 Hz)	2.40 (d; J=12.75 Hz) 2.24 (ddd/dt; J=4.12, 12.35, 16.80, 24.7 Hz)	-
8	-	-	-	-	(6.2. br s)
9	-	-	1.73	1.73	-
10	-	-	-	-	-
11	-	-	1.82 (m) 1.67 (m)	1.82 (m) 1.66 (m)	-
12	-	-	2.29 (m) 2.59 (sept/m; J=3.35, 5.80, 8.65, 14,45 Hz)	2.29 (m) 2.57 (sept/m; J=3.35, 5.80, 8.65, 14,45 Hz)	-
13	-	-	-	-	-
14	-	-	5.86 (s)	5.85 (s)	(6.89, d, J-12 Hz)
15	-	-	-	-	(7.38, d, J-12 Hz)
16	-	-	4.72 (s) 4.70 (s)	4.73 (s) 4.71 (s)	
17	-	-	4.90 (s) 4.49 (s)	4.88 (s) 4.77 (s)	
18	0.68 (3H,s)	0.70 (3H,s)	1.16 (s)	1.1 (s)	
19	1.01(3H,s)	1.01(3H,s)	-	-	
20	-	-	0.74 (s)	0.69 (s)	
21	0.92 (3H, d, 6.4)	1.02 (3H, d, 7.5)			
22	-	5.14 (dd, 8.4, 8.8)			
23	-	5.02 (dd, 8.4, 8.4)			
24	-	-			
25	-	-			
26	0.81 (3H, d, 6.5)	0.79 (3H, d. 6.5)			
27	0.83 (3H, d, 6.5)	0.85 (3H, d, 6.5)			
28	-	-			
29	0.85 (3H, t, 7.5)	0.80 (3H, t, 7.5)			

Table 1: ^{1}H-NMR spectral data for Compounds (**1-5**).

Position	δ_c, Multi				
	1	2	3	4	5
1	37.3 t	37.3 t	38.0 t	39.2 t	
2	31.7 t	31.5 t	18.3 t	21.5 t	123.8
3	71.8 d	71.8 d	37.0 t	35.4 t	103.8
4	42.3 t	42.3 t	47.4 s	56.3 s	183.9
5	140.8 s	140.8 s	49.4 d	56.4 s	166.7
6	121.7 d	121.7 d	26.8 t	27.2 t	100.3
7	31.9 t	31.9 t	37.7 t	38.6 t	166.4
8	31.9 d	31.7 d	147.1 d	115.3 s	95.1
9	51.2 d	51.3 d	56.2 d	65.1 s	159.4
10	36.5 s	36.5 s	39.0 s	39.7 s	105.2
11	21.1 t	21.1 t	21.2 t	24.5 s	114.1
12	39.8 t	39.7 t	27.5 t	27.5 t	147.0
13	42.2 s	42.2 s	170.9 s	147.5 s	151.0
14	56.8 d	56.8 d	115.2 s	115.3 t	116.8
15	24.3 t	24.3 t	174.2 s	171.0 s	120.4

16	28.2 t	28.2 t	73.1 t	73.2 d	
17	56.1 d	56.1 d	107.2 t	106.8 t	
18	12.0 q	11.8 q	16.3 q	19.0 q	
19	19.4 q	19.8 q	184.8 s	174.2 t	
20	36.1 d	40.5 d	14.7 q	15.3 d	
21	18.8 q	21.2 q			
22	34.0 t	138.3 d			
23	26.1 t	129.3 d			
24	45.9 d	50.2 d			
25	29.2 d	31.7 d			
26	19.0 q	21.1 q			
27	19.0 q	19.0 q			
28	29.1 t	25.4 t			
29	12.2q	12.0 q			

Table 2: ^{13}C-NMR spectral data of Compounds (1-5).

Group	Glucose(mmol/L) 0 day	Glucose(mmol/L) 14 day	Glucose(mmol/L) 28 day
T2WC (n=6)	7.57 ± 0.52 (100%)	8.30 ± 0.88 (110%)	8.52 ± 1.43 (113%)
Gliben (n=6)	7.99 ± 1.03 (100%)	6.59 ± 1.30 (82%)	6.39 ± 1.14* (80%)
Ethanol ext (n=6)	8.86 ± 0.79 (100%)	7.17 ± 1.22 (81%)	6.42 ± 1.32* (72%)

Results are expressed as Mean ± SD; *p<0.05 vs Type 2 water control (ANOVA with post Hoc Bonferroni test). T2WC=Type 2 Water Control, Gliben=Glibenclamide.

Table 3: Effect of *P. thyrsiflorus* flower extract on fasting blood glucose level of STZ-induced Type 2 diabetic model rats.

Results and Discussion

Chemistry of *P. thyrsiflorus*

Repeated chromatographic separation and purification of dichloromethane and 1-butanol part of methanol extracts of flowers of *P. thyrsiflorus* provided five compounds (**1-5**). The ^{13}C-NMR (Table 2) of **1** showed 29 carbons including an oxymethine carbon signal at δ_C 71.8 and two olefinic carbons at δ_C 140.8 and δ_C 121.7 characteristic of spirostene [11]. The multiplicities of the carbon signals were determined by DEPT 135 experiments which revealed the presence of 6 methyls, 11 methylenes, 9 methines and 3 quaternary carbon atoms in compound **1**. The ^{1}H- (Table 1) and ^{13}C- (Table 2) NMR spectral data of compound **1** are similar to the reported data for *β*-sitosterol [12,13]. Therefore, the structure of compound **1** is characterized as *β*-sitosterol (Figure 1). *β*-Sitosterol is a very common steroidal compound present in almost all kinds of plants. *β* -Sitosterol is reported for the first time from the flowers of this plant.

The ^{1}H-NMR spectral data (Table 1) of compound **2** revealed multiplet signal for an oxymethine proton at δ_H 3.52. The olefin proton resonating at δ_H 5.35 was charecterististic of 5-steroids [11]. The spectrum revealed signals at δ_H 0.68 and 1.01 (3H each) assignable to two tertiary methyl groups at C-18 and C-19, respectively. The signals of two further secondary methyl groups at δ_H 0.84 (*J*=7.3 Hz) and δ_H 0.83 (*J*=7.3 Hz) could be attributed to two methyl groups at C-26 and C-27, respectively. The doublet at δ_H 0.92 (d, *J*=8.0 Hz, H-21) was demonstrative of a methyl group at C-21. On the other hand, the three-proton triplet at δ_H 0.81 could be assigned to the primary methyl group at C-29. The ^{1}H- (Table 1), ^{13}C- (Table 2) and DEPT-NMR spectra of compound **2** are similar to the reported data of stigmasterol [12,13]. Therefore, the structure of compound **2** is characterized as stigmasterol. Stigmasterol is very common steroidal compound present in many plants but stigmasterol is the first report from the flowers of this plant.

Compound **3** is a diterpenoid compound. Its IR (KBr) ν: 2940 (-OH), 1691 (>C=O), 1528, cm^{-1} spectrum indicated the presence of hydroxyl and carbonyl groups, and carbon carbon double bonds in the compound **3**. The molecular formula $C_{20}H_{28}O_4$ of **3** was deduced from GCMS data. Three CH_2 proton appears at δ_C 1.63 (m), 1.67(m), 1.78 (m). Two H appears at δ_H 4.70 (s) and 4.72 (s) for the CH_2 of furan ring. Two olefinic H of 17 carbon appears at δ_H 4.90 (s) and 4.49 (s). Another olefinic H of furan ring comes at δ_H 5.86 (s). Carboxylic functional group containing carbon appears at δ_C 184.8, furan ring containing quarternary carbon at δ_C 170.9 and cyclic carbonyl carbon at δ_C 174.2. The ^{1}H-, ^{13}C- and DEPT-NMR spectra of compound **3** are similar to the reported data of 8(17),13-labdadien-15,16-olide-19-oic acid [9,14]. Therefore, the structure of compound **3** is characterized as 8(17),13-labdadien-15,16-olide-19-oic acid. This is the first report of isolation of 8(17),13-labdadien-15,16-olide-19-oic acid from the flowers of this plant.

The molecular formula $C_{20}H_{30}O_4$ of compound **4** was deduced from GCMS data. Compound **4** has three CH_2 protons appearing at δ_H 1.65 (m), 1.69 (m) and 1.78 (m). Two H appears at δ_H 4.71 (s) and 4.73 (s) for the CH_2 of furan ring. Two olefinic H of 17 carbon appears at δ_H 4.88 (s) and 4.77 (s). Another olefinic H of furan ring comes at δ_H 5.85 (s). Hydroxyl functional group containing carbon appears at δ_C 174.2, furan ring containing quarternary carbon at δ_C 147.5 and cyclic carbonyl carbon at δ_C 171.0. The ^{1}H- (Table 1), ^{13}C- (Table 2) and DEPT-NMR spectra of compound **4** are similar to the reported data of 8(17),13-labdadien-15,16-olide-19-oic acid [9,15] except the carboxylic acid functional group reduced to hydroxyl group. Therefore, the structure of compound **4** is characterized as 19-hydroxy-8(17),13-labdadien-15,16-olide. This is first report of 19-hydroxy-8(17),13-labdadien-15,16-olide from the flowers of this plant.

The molecular formula $C_{15}H_{10}O_6$ was determined from 1D and 2D NMR data of compound **5**. The ^{13}C-NMR spectrum of **5** displayed 15 carbon atoms, which is characteristic of a flavonoid type skeleton. The molecular mass of this compound is 286 g/mol. The ^{1}H- (Table 1), ^{13}C- (Table 2) and DEPT-NMR data of compound **5** are similar to

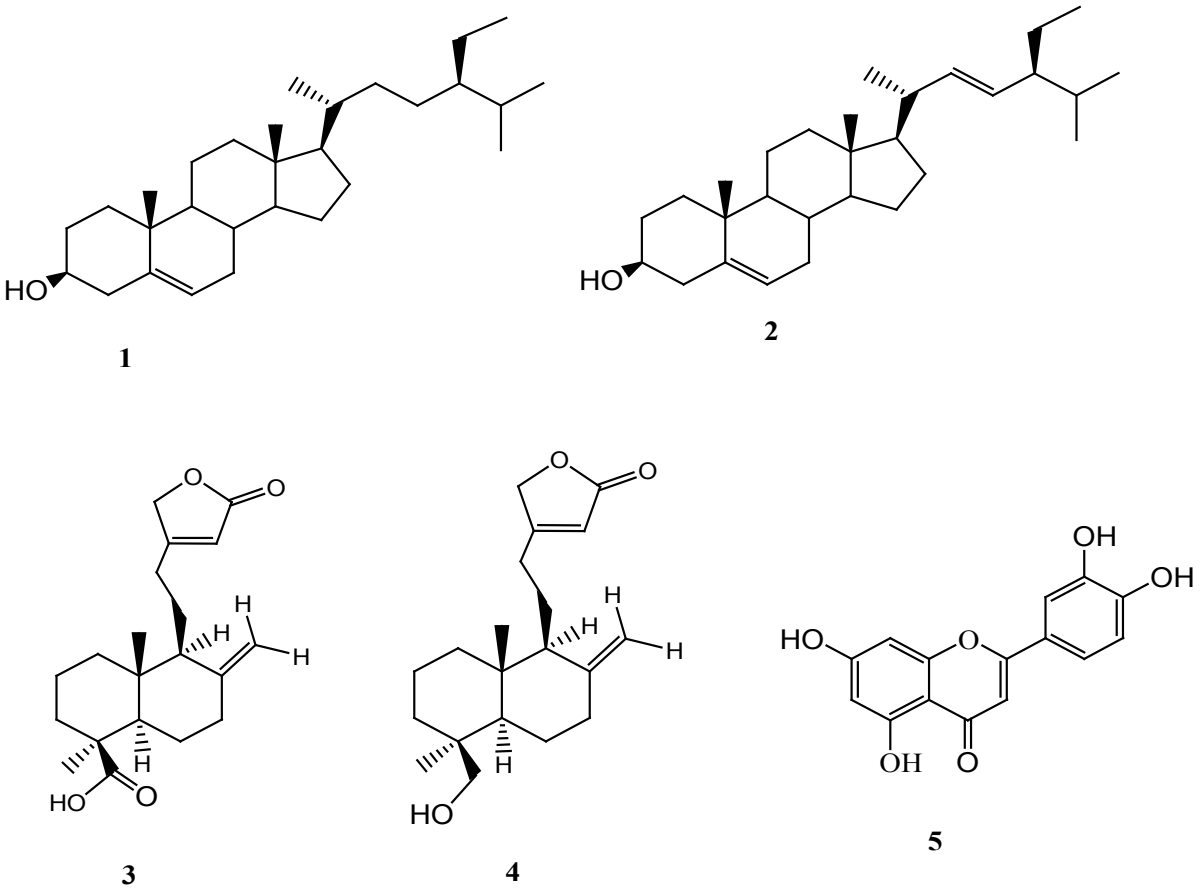

Figure 1: Structure of isolated 05 compounds from *P. thyrsiflorus*.

the reported data of luteolin [16,17]. On the basis of all the spectral data, the compound **5** is characterized as luteolin. Luteolin is a known compound and its IUPAC name is 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone. Compound **5** is isolated for the first time from this plant.

Antidiabetic effects of *P. thyrsiflorus* flowers

Effect of *P. thyrsiflorus* on fasting serum glucose level of Type 2 diabetic model rats: Fasting serum glucose (FSG) levels of type 2 diabetic models rats of the three experimental groups were almost similar on 0 day (Table 3). After oral administration of respective treatment to the type 2 diabetic model rats of different groups for 28 days of experimental period, it was found that the FSG level of type 2 rats treated with ethanol extract of *P. thyrsiflorus* flowers showed a significant decrease compared to that of control group ($p=0.042$). Morever, It is seen from the Table 3 that ethanol extract treated group showed a 28% reduction of FSG level compared to 0 day value. Chakravaty *et. al.* found anti-hyperglycemic activity with *P. thyrsiflorus* flower on STZ induced diabetic mice [10]. As expected, glibenclamide also ameliorated the diabetic condition on 28th day. Glibenclamide significantly ($p=0.037$) reduced fasting glucose on 28th day when compared to that of control group. On the contrary, there was a 13% increase in the fasting glucose level of type 2 control group on 28th day of experimental period compared to the baseline.

Effect of *P. thyrsiflorus* flower on serum insulin level of STZ-induced Type 2 diabetic model rats: The effect of *P. thyrsiflorus* flowers extract on insulinemic status of type 2 diabetic model rats was observed (Figure 2). At the beginning of the study period, all groups showed almost similar serum insulin level. After 28 days consecutive feeding glibenclamide treated rats showed more than fivefold increase in serum insulin level which was highly significant ($p<0.001$). Ethanol extract showed a 23% increase in serum insulin level at the end of the study period compared to baseline level, however the increase was not significant. As it is seen from the table, T2WC group showed a 48% reduction compared to baseline value which is logical. Type 2 diabetic rat model those are produced in this study by injecting STZ to neonate rats have hypoinsulinemia which resemble beta cell secretory defect. Therefore, in this study the condition of the Type 2 water control group deteriorates with time which is manifested by hyperglycemia and decreased serum insulin level.

Effect of *P. thyrsiflorus* flowers extract on the body weight of Type 2 diabetic model rats: The effect of *P. thyrsiflorus* flowers extract on body weight of type 2 diabetic model rats was observed during 28 days study period. Body weight of each rat was taken at seven days interval. No significant change was found in body weight in any group after 28 days of study (Table 4).

Effects of *P. thyrsiflorus* flowers extract on serum cholesterol and triglyceride levels of Type 2 diabetic model rats: Effect of *P. thyrsiflorus* flowers extract on serum cholesterol and triglyceride level

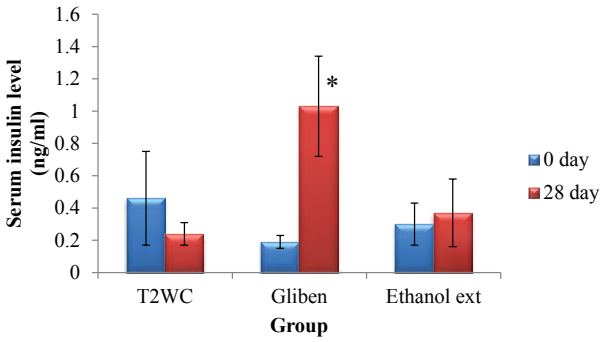

Figure 2: Effect of *P. thyrsiflorus* flower extract on the serum insulin level of STZ- induced Type 2 diabetic model rats. *p<0.001 compared water control group (ANOVA with post Hoc Bonferroni test).

Group	Body weight (g)				
	0 day	7 day	14 day	21 day	28 day
T2WC (n=6)	192 ± 21 (100%)	185 ± 28 (96%)	188 ± 32 (98%)	182 ± 29 (95%)	193 ± 34 (101%)
Gliben (n=6)	182 ± 13 (100%)	189 ± 25 (104%)	183 ± 8 (101%)	182 ± 7 (101%)	182 ± 16 (100%)
Ethanol ext (n=6)	202 ± 7 (100%)	193 ± 19 (96%)	200 ± 18 (99%)	195 ± 20 (97%)	198 ± 18 (98%)

Results are expressed as Mean ± SD. ANOVA with post Hoc Bonferroni test was done.

Table 4: Effect of *P. thyrsiflorus* flowers extract on the body weight of Type 2 diabetic model rats.

Group	Cholesterol (mg/dL)		TG (mg/dL)	
	0 day	28 day	0 day	28 day
T2WC (n=6)	79 ± 5.93 (100%)	73 ± 8.28 (92%)	70 ± 4.12 (100%)	59 ± 8.56 (84%)
Gliben (n=6)	81 ± 8.87 (100%)	65 ± 5.78 (80%)	77 ± 7.53 (100%)	59 ± 11.85 (77%)
Ethanol ext (n=6)	80 ± 11.21 (100%)	71 ± 8.96 (89%)	70 ± 9.09 (100%)	54 ± 7.52 (77%)

Results are expressed as Mean ± SD. Statistical analysis was done using ANOVA with post Hoc Bonferroni test.

Table 5: Effects of *P. thyrsiflorus* flowers extract on serum cholesterol and triglyceride levels of STZ-induced Type 2 diabetic model rats.

Group	HDL (mg/dL)		LDL (mg/dL)	
	0 day	28 day	0 day	28 day
T2WC (n=6)	40 ± 2.81 (100%)	33 ± 3.25 (83%)	25 ± 6.98 (100%)	28 ± 10.62 (112%)
Gliben (n=6)	40 ± 3.08 (100%)	36 ± 6.26 (90%)	26 ± 7.07 (100%)	17 ± 9.70 (65%)
Ethanol extract (n=6)	41 ± 3.20 (100%)	41 ± 3.39* (100%)	25 ± 13.60 (100%)	19 ± 9.05 (76%)

Results are expressed as Mean ± SD. *p<0.05 vs Type 2 water control (ANOVA with post Hoc Bonferroni test).

Table 6: Effect of *P. thyrsiflorus* flowers extract on the serum HDL-C and LDL-C levels of STZ-induced Type 2 diabetic model rats.

is presented in Table 5. Ethanol extract of *P. thyrsiflorus* flowers caused non-significant reduction in total cholesterol level on 28th day [Serum cholesterol (M ± SD) mg/dl, 0 day (80 ± 11.21) vs. 28 day (71 ± 8.96)] (p<NS), Glibenclamide decreased serum cholesterol level by 20% when compared to the base line level. Type 2 control also showed 8% decrease of serum cholesterol level. In case of serum triglyceride (TG) level, there was a reduction of 23% in glibenclamide treated groups after 28 days study period. Ethanol extract treated group also showed a reduction in serum TG level by 16% (Table 5), although the reduction level was not significant.

Chronic effects of *P. thyrsiflorus* flowers extract on serum HDL-C and LDL-C levels of STZ-induced Type 2 diabetic model rats: The effects of chronic treatment with extract of *P. thyrsiflorus* flowers on the serum HDL- and LDL-cholesterol levels of type 2 diabetic model rats are summarized in Table 6. It was found that at the end of the 28 days study period, HDL level increased significantly (p=0.031) in ethanol extract of *P. thyrsiflorus* treated group compared to water control group. Glibenclamide treated group was unable to increase serum HDL level at the end of 28 days study period when compared to the water control group. As it is seen from the table water control group had a 17% decrease in HDL level compared to its baseline value. In case of atherogenic LDL-cholesterol, the level was decreased by 24% with the treatment of ethanol extract of *P. thyrsiflorus* flowers (25 mg/dL on the 0 day to 19 mg/dL on the 28th day) at the end of the study period. Glibenclamide also decreased LDL level by 35% compared to the initial value. However, the decrease was not statistically significant for both the cases. On the other hand, control group showed a 12% increase in serum LDL which is harmful for health. Type 2 diabetes is associated with marked imbalance in lipid metabolism [18]. As we know, the most vulnerable problem in dyslipidemia is the increase in atherogenic lipids i.e., increase in serum LDL-C, Triglyceride and total cholesterol levels with the reduction in good cholesterol (HDL-C) level [19-21]. It is now well established that elevated triglycerides and low high-density lipoprotein cholesterol (HDL-C) levels contribute to cardiovascular disease risk. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipophytic hormones on the fat depots mainly due to the action of insulin. The role of hypolipidemic agents is to correct dyslipidemia. In this study, at the end of the experimental period, ethanol extract of *P. thyrsiflorus* flowers decreased Total cholesterol, triglycerides and LDL-C level although non-significantly and significantly improved HDL-C level (p=0.031) compared to water control group of Type 2 rats. This implies that ethanol extract of *P. thyrsiflorus* flowers can prevent or be helpful in reducing the atherogenic lipids in diabetes.

Conclusion

From the obtained results it may be concluded that, flowers of *P. thyrsiflorus* possesses hypoglycemic and to some extent hypolipidemic properties. The flowers of the plant contained 5 compounds which have been isolated and characterized. The obtained antidiabetic activity by the ethanolic extract of *P. thyrsiflorus* flowers may be due to the presence of these compounds, the activities of which remain to be explored in future.

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