Effect of compounds identified in the active fraction of *pericampylus glaucus* on blood glucose and lipid profiles in streptozotocin-induced diabetic rats

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Received 5 July 2016
Accepted 6 September 2016

Egyptian Pharmaceutical Journal
2017, 16:8–15

Objective
The present study was designed to determine the effect of compounds identified in the active fraction of Pericampylus glaucus on blood glucose and lipid profiles in streptozotocin (STZ)-induced diabetic rats.

Materials and methods
Initially, the antidiabetic activity of petroleum ether, n-hexane, chloroform, and ethanolic extracts of *P. glaucus* was evaluated in STZ-induced diabetic rats at a dose of 400 mg/kg body weight. Then, the potential extract was fractionated by different solvent systems to obtain various fractions. Next, fractions were investigated again at a dose of 100 mg/kg body weight to find the active fraction. The active fraction was examined using STZ-induced diabetic rats for 21 days. The blood glucose levels were observed weekly and various biochemical parameters were determined on the day the rats were killed.

Results
The active fraction was subjected to gas chromatography mass spectrometry to find the compounds present in the active fraction of ethanolic extract of *P. glaucus*. The ethanolic extract was noted to have significant ($P<0.001$) antidiabetic activity in diabetic rats compared with the other extracts. Four fractions (FA, FB, FC, and FD) were collected from the active ethanolic extract. Among these, fraction B, which was collected from a mixture of petroleum ether and ethyl acetate, was found to have a high ($P<0.001$)-attenuation effect on blood glucose levels compared with the others, except for 'fraction D' ($P<0.01$), which was collected from ethanol. The data showed that the active fraction (fraction B) also induced significant ($P<0.05$, <0.01, <0.001) attenuation in the levels of triglyceride, total cholesterol, and LDL and a significant ($P<0.01$) improvement in the level of HDL. In gas chromatography mass spectrometry analysis, 10 peaks were noted, which suggests the presence of 10 compounds in the active fraction responsible for the blood glucose-lowering effect and biochemical parameters.

Conclusion
The present study confirmed the presence of compounds identified in *P. glaucus* responsible for the attenuation of blood glucose and lipid parameters.

Keywords:
active fraction, antidiabetic, broad fractions, ethanolic extract, 10 peaks, *Pericampylus glaucus*

Egypt Pharmaceut J 16:8–15

1687-4315

Introduction

Diabetes mellitus is a chronic metabolic syndrome that involves variations in carbohydrates, urine, and fat metabolism [1]. There are two main types of diabetes mellitus, for example, type I, insulin-dependent diabetes mellitus and type II non-insulin-dependent diabetes mellitus [2]. According to the IDF statistical report, 382 million individuals worldwide have diabetes and 592 million (10%) of the entire population will be affected in 2035 [3].

A large number of Malaysian been suffered from diabetes and almost became 3.3 million. A population-based survey has reported approximately double the number of diabetic patients over the duration ranging from 1985 to 2006 [4]. The WHO has projected that by 2030, an estimated 2.48 million Malaysians will have diabetes [1]. However, at present, about 3.3 million Malaysians have diabetes, which is higher than the estimation for 2030. The prevalence of diabetes in such ratio, it is essential to implement policies aimed at improved human healthcare. The use of natural remedies for the treatment of diabetes has a long history. The genus

Pericampylus glaucus belongs to the family *Menispermaceae*, found commonly in Malaysia [1]. Various parts of the plant are used traditionally for the treatment of various diseases like productive cough, cold, relief of dysarthria, fever, joint pain, muscle pain, diabetes, constipation, treatment of snake bites, and abdominal distention [1]. The plant was previously reported to have radical scavenging [5], non toxic and lethal up to dose 4000 mg/kg (b.wt) in experimental animals [1], and anticancer effects [6], for the treatment of AIDS, hepatitis B virus, and hepatitis C virus [7], and antidiabetic properties [8]. Therefore, the present study aimed to determine the antidiabetic and antihyperlipidemic activities of *P. glaucus* against streptozotocin (STZ)-induced diabetic rats and identification of compounds.

Materials and methods

Collection and extraction of plant material

The leaves of *P. glaucus* were collected and authentication was carried out from FRIM, Malaysia [specimen herbarium number (FRIM/394/490/5/18 (118)]. The leaves were dried in the shade and were ground into a coarse powder.

The powder (500 g) was then extracted by continuous hot extraction using the Soxhlet apparatus at a temperature of 78°C using different solvents according to their polarity, that is, petroleum ether, followed by n-hexane, chloroform, and ethanol. The various extracts were then concentrated under reduced pressure through a rotary evaporator (N-10000 Eyela; Tokyo Rikakikai Co. Ltd, Tokyo, Japan) and preserved in a desiccator for further analysis.

Drugs and chemicals

Glibenclamide and STZ were purchased from Sigma-Aldrich Bio Syn Tech Malaysia (Selangor, Malaysia). Analytical grades of chemicals and various solvents for extraction were used (E. Merck, Menara Sunway Annexe, Petaling Jaya, Selangor; Astral Laboratory Chemicals, Mangrove Lane Taren Point NSW 2229 Australia, R/M Chemical, Barotiwala, Himachal Pradesh 173201, India, and Sigma-Aldrich Co., Bio Syn Tech Malaysia Group Sdn Bhd). The biochemical parameters were determined using an analyzer model (BA-D200A).

Test animal

Adult Sprague-Dawley rats (90–110 g) were used and were kept in the animal holding facility, Department of Pharmacology, Lincoln University College, Malaysia. The experimental procedures used were approved by the Lincoln University College, Malaysia, Animal Ethics Committee (LUC-AEC) with reference LUC-AEC number PHARM/2013/MSM/02-August/11/September 2014-August 2016, after confirming that the proposed research work complied with the Malaysian regulation.

Induction of diabetes mellitus

In the animal model, diabetes was induced by a single dose of STZ 50 mg/kg body weight (intraperitoneally), dissolved in freshly prepared 0.01 mol/l citrate buffer, pH 4.5. After 72 h, animals with blood glucose levels higher than 14 mmol/l were selected for experiments [8].

Effect of various extracts on blood glucose level in streptozotocin-induced diabetic rats

The effect of various extracts on blood glucose level was determined using the method of Macharla *et al.* [9], with some modifications. The diabetic animals were divided into six categories, six animals in each group. The first group was the untreated diabetic group. The second, third, fourth, fifth, and sixth groups received petroleum ether, n-hexane, chloroform, ethanolic extract, and standard at a dose of 400 and 20 mg/kg (body weight) postoperatively. The blood samples were collected from the tail vein of tested animals at 0, 2, 4, and 6 h after the administration of various extracts. The blood glucose level was determined using the glucose oxidize peroxidase method with glucose oxidase strips [10].

Fractionations of active crude extract in different solvent systems

The effective ethanolic extract of *P. glaucus* (20 g) was subjected to fractionation. The fractionation was carried out through chromatography over a column of stationary silica gel with a mesh size of 230–400 in different solvent systems. The column was loaded up to the level of three-fourth of the total column with a slurry of silica gel with a 230–400 mesh size that was admixed with a nonpolar n-hexane solvent. The ethanolic plant extract was combined with equivalent amounts of silica gel and dried completely [11]. The column was effectively eluted through different solvent systems, such as n-hexane, followed by chloroform, petroleum ether, ethyl acetate, acetone, and ethanol in different concentration ratios such as n-hexane (100%), n-hexane–chloroform mixture (30 : 70), chloroform–petroleum ether mixture (30 : 70), petroleum ether and ethyl acetate mixture (70 : 30), ethyl acetate and acetone mixture (70 : 30), and finally 95% ethanol 100 v/v%.

Effect of various fractions on blood glucose levels in streptozotocin-induced diabetic rats

The collected fractions were also assessed to determine the glucose-lowering activity in STZ-induced diabetic animals according to the process that was described by Islam *et al.* [12], with some modifications.

The Sprague-Dawley rats were divided into six groups of four animals each. The first group was the normal control group. The second group was administered glibenclamide at a dose of 0.002 g/kg (body weight), whereas the third, fourth, and fifth groups received various fractions (A, B, C, and D) at a dose of 100 mg/kg body weight. The blood samples were collected from the tail vein at 0, 2, 4, and 6 h and the blood glucose levels were determined using glucose oxidize reactive standard strips [10].

Effect of the active fraction on blood glucose and lipid profile in streptozotocin-induced diabetic rats

The effect of the active fraction on blood glucose levels and lipid profiles were determined according to the technique that was reported by ([11]), with some modifications [13]. The normal and STZ-induced diabetic rats were divided into four groups of six animals each. Group 1 was the control group. The diabetic animals in group 2 were the untreated diabetic group. The diabetic animals in group 3 were treated with the standard glibenclamide drug at a dose of 20 mg/kg. The fourth group was treated with the active fraction at a dose of 50 mg/kg body weight. All the investigated animals were administered the tested drug for a period of 21 days by oral gastric gavages.

Identification of compounds in the active fraction of *P. glaucus* through gas chromatography mass spectrometry analysis

The identification of compounds present in the active fraction of *P. glaucus* was carried out by a direct comparison of retention time and mass spectra using the record library combined with the NIST Library.

Statistical analysis

Statistical analysis was carried out as mean±SEM, followed by a two-way analysis of variance test using Graph Pad Prism software (Graph Pad Software, Inc. 7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA), and for multiple-comparison tests among the groups, the Bonferroni test was performed. A probability level of P less than 0.05 was considered statistically significant.

Results

The present research aimed to establish the pharm acological effects of identified bioactive components from the active fraction of *P. glaucus* on blood glucose levels and lipid profiles in STZ-induced diabetic rats.

Effect of various extracts on blood glucose levels in streptozotocin diabetic rats

The effect of various extracts of *P. glaucus* on blood glucose levels in STZ-induced diabetic rats was measured at 0, 4, and 6 h and was matched to untreated diabetic animals to determine the best active extract that can be used for further studies. No significant attenuations in blood glucose levels were noted in all the groups treated with extracts for the first 2 h, except for the ethanolic extract. The attenuations in blood glucose levels were found to be higher with the crude ethanolic extract, which started after 2 h of treatment and became significant ($P<0.001$) for up to 6 h compared with the groups treated with other extracts and the diabetic group. The other extracts, except n-hexane for petroleum ether and chloroform extract, also induced a slight ($P<0.05$) reduction in blood glucose levels. The ethanolic extract induced a 16.20% decrease in blood glucose compared with the untreated diabetic group (Fig. 1).

Fractionation of the active crude extract by column chromatography

After fractionation, four fractions were collected and were assigned the following names: 'fraction A for chloroform: petroleum ether mixture', 'fraction B for petroleum ether: ethyl acetate mixture', 'fraction C for ethyl acetate and acetone mixture,' and 'fraction D for ethanol'. These fractions were investigated further to determine the active fraction responsible for attenuation of blood glucose and lipid profiles.

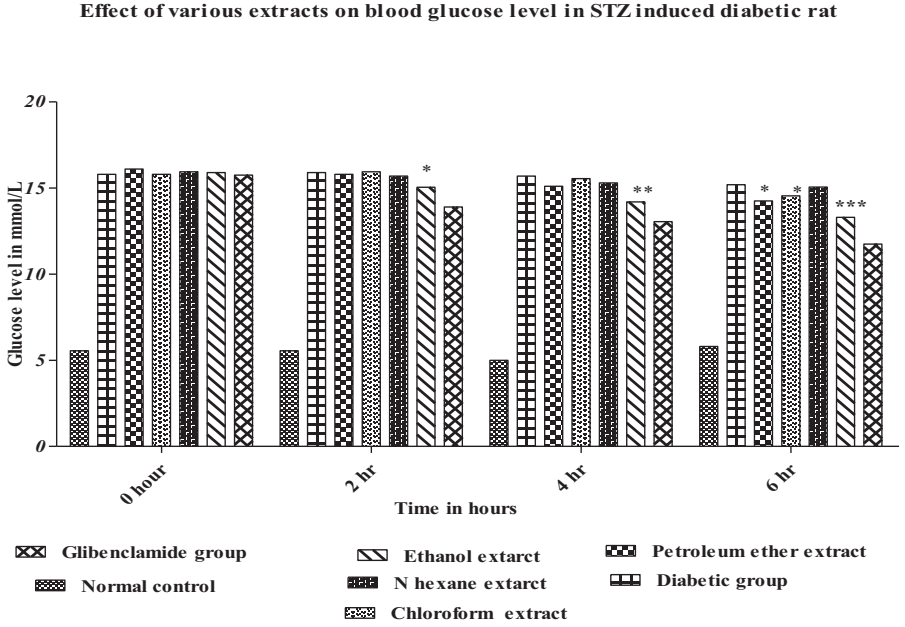
Effect of various fractions on blood glucose levels in streptozotocin-induced diabetic rats

The effect of various fractions (A, B, C, and D) was examined in STZ-induced diabetic rats. In all animals treated with various fractions at a dose of 100 mg/kg (body weight), the blood glucose level remained the same ($P>0.05$) for the first 2 h. A slight reduction in the blood glucose level (14.72±0.02 mmol/l; $P<0.05$) was noted at 4 h of treatment, with fraction B showing a significant difference ($P<0.001$; 14.05±0.05 mmol/l) at 6 h compared with the untreated diabetic control group. The percentage of decrease in glucose was 5.94% at 4 h and 9.38% at 6 h in the tested group that received fraction B at 100 mg/kg (body weight). Fraction D also induced significant attenuations in blood glucose level ($P<0.01$; 15.15±0.05 mmol/l) (2%) at 6 h, respectively, compared with the untreated diabetic control group (Fig. 2).

Effect of the active fraction on blood glucose level and lipid profile in streptozotocin-induced diabetic rats (21 days)

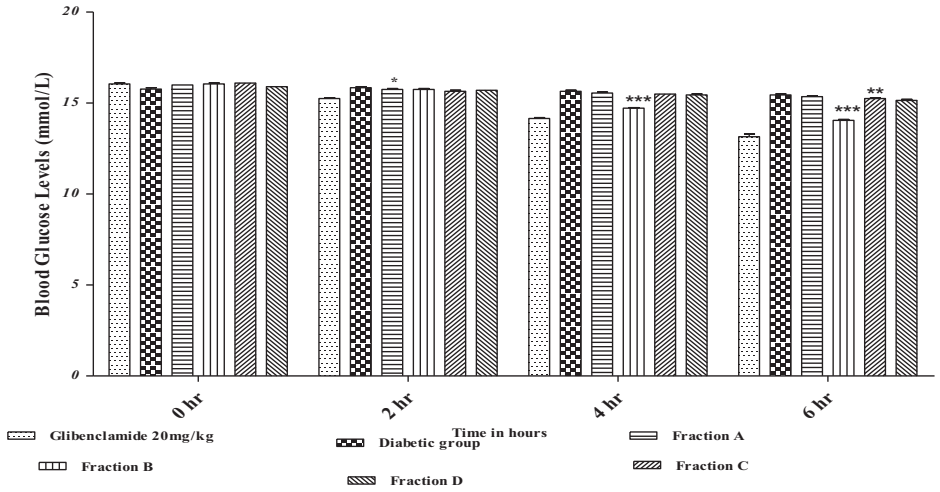
The effects of the oral active fraction (fraction B) at a dose of 50 mg/kg body weight, along with the standard drug on blood glucose level and lipid profiles in STZ-induced diabetic rats at 0, 7th, 14th, and 21st day, are shown in Fig. 3. A significant increase in blood sugar levels was noted in animals receiving STZ at 50 mg/kg (body weight). The active fraction (fraction B)-treated animals at a dose level of 50 mg/kg showed a significant reduction ($P<0.001$) in blood glucose levels compared with the diabetic control group. The reduction

Figure 1



Effect of various extracts on blood glucose levels in STZ-induced diabetic rats. The values are expressed as mean±SEM (N=5), *P<0.05, **P<0.001, ***P<0.0001 significant compared with the diabetic control groups. The statistical test 'two-way analysis of variance' was applied, followed by the Bonferroni test.

Figure 2



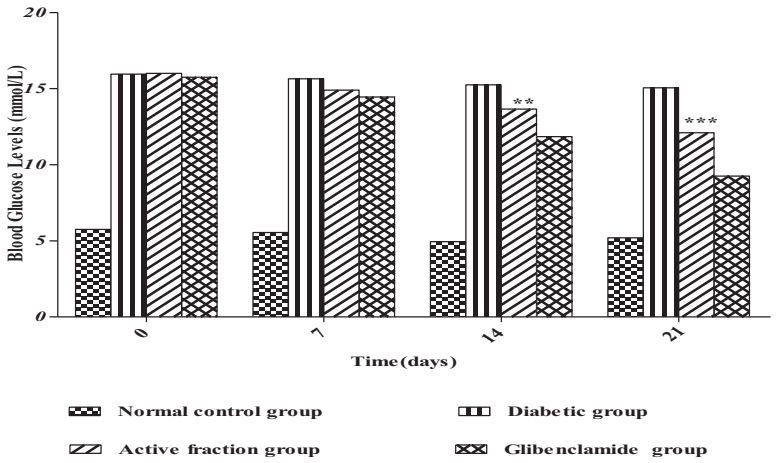
Effect of various fractions on blood glucose on STZ-induced diabetic rats. Data are expressed as mean±SEM (N=4). *P<0.05, **P<0.01, ***P<0.001 significant compared with the diabetic group. The statistical test 'two-way analysis of variance' was applied, followed by the Bonferroni test.

in blood glucose level through the active fraction (fraction B) in tested animals was found to be significant (P<0.001): from 16.00±0.20, 14.90±0.15, and 11.65±0.14 mmol/l at 0, 7, and 14 days of treatment to 12.45±0.15 mmol/l before the animals were killed compared with the diabetic control group (Fig. 3).

Effect of the active fraction and standard on lipid profiles in streptozotocin-induced diabetic rats

The effects of the active fraction (fraction B) that was collected from the leaves of a crude ethanolic extract of *P. glaucus* and the standard drug glibenclamide on lipid profiles in STZ-induced diabetic animals are summarized in Table 1. A significant (P<0.001) increase in total triglycerides, LDL, total cholesterol, creatinine, and urea and a reduction in HDL were found in the diabetic control group. The tested animals that received the active isolated fraction orally at a dose of 50 mg/kg body weight for 20 days showed significant (P<0.001) attenuations in the level of total cholesterol, triglycerides, urea, and LDL, and a significant (P<0.05) increase in HDL, but a nonsignificant (P>0.05) increase in creatinine compared with

Figure 3



Effect of the active fraction on fasting blood glucose levels in STZ-induced diabetic rats. Data are expressed as mean±SEM (N=6), $^{**}P<0.01$, $^{***}P<0.001$ significant compared with the diabetic control group; The statistical test used was one-way analysis of variance, followed by the Bonferroni test.

Table 1 Effect of the active fraction of *Pericampylus glaucus* and glibenclamide on lipid profiles in streptozotocin-induced diabetic rats

Serum lipids profile	Total cholesterol	Triglyceride (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)
Diabetic group	6.75±0.25	7.95±0.05	5.10±0.15	0.63±0.08	3.22±0.40	1.10±0.14
Normal group	1.55±0.15	4.50±0.20	2.25±0.05	2.30±0.10	1.80±0.10	0.07±0.01
Diabetic+active fraction	4.35±0.05***	4.75±0.25***	3.80 ±0.30**	1.95±0.05**	2.70±0.15	0.40±0.10
Diabetic+glibenclamide	2.90±0.20***	3.90±0.30***	2.20±0.10***	2.30±0.2***	2.10±0.10**	0.07±0.02

Data are expressed as mean±SEM (N=6). $^{*}P<0.05$. $^{**}P<0.01$. $^{***}P<0.001$ significant compared with the diabetic control; the statistical test used was two-way analysis of variance, followed by the Bonferroni test.

the untreated diabetic control group. The total triglyceride levels attenuated to 4.75±0.25 mmol/l in the diabetic group, which received the active fraction at a dose of 50 mg/kg from 7.95±0.05 mmol/l. The total cholesterol levels were obse rved to attenuate to 4.35±0.05 mmol/l in the diabetic group that received the active isolated fraction at 50 mg/kg body weight as compared to untreated diabetic group (6.75±0.25 mmol). The HDL levels were found to increase from 0.63±0.08 mmol/l in the diabetic group that received the active fraction at 50 mg/kg body weight to the 1.95±0.05 mmol/l treated group compared with the untreated diabetic control group.

Identification of phytochemical compounds identified in an active sample of *Pericampylus glaucus*

The active fraction, fraction B (petroleum ether and ethyl acetate), collected from the active crude ethanolic extract of *P. glaucus*, indicated 10 peaks through gas chromatography mass spectrometry (GCMS), which showed the presence of 10 either active or inactive phytoconstituents. The compounds identified in the active fraction sample of *P. glaucus* on the basis of mass spectrum are presented in Fig. 1. In terms of the relationship of the mass spectrum range of the components with the record library, 10 phytocomponents were identified. The names of the identified compounds, their molecular weight, along with their retention time, molecular formula, and their concentration (peak%), are tabulated in Table 2 (Fig. 4).

Discussion

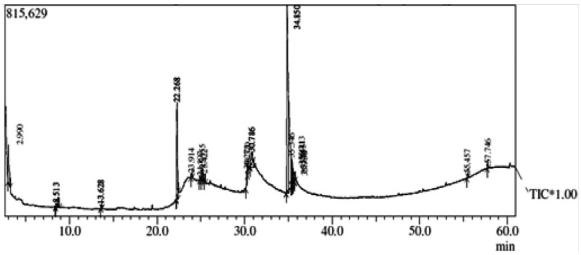
At present, the importance of investigations of plant-based medicine is growing, particularly as a part of drug innovation programs. Our earlier research works proved the attenuation in blood glucose and lipids profiles with the crude ethanolic extract *P. glaucus*. On the basis of the preceding research, here, an attempt was made to identify the compounds from the active fraction of *P. glaucus* responsible for reductions of blood glucose and lipid profiles.

In animals treated with various crude plant extracts, the crude ethanolic extract was noted to induce a significant ($P<0.001$) blood glucose-lowering effect as compared to other extracts (petroleum ether, n-hexane, chloroform) and untreated diabetic control group. The petroleum ether and chloroform extract also induced a slight ($P<0.05$) decrease in blood glucose levels. However, was not significant as it was produced by ethanolic extract.

Table 2 List of compounds identified in the active sample fraction of Pericampylus glaucus

Serial number	List of compounds identified	m/z	Retention time	Area	S.I.	Molecular weight	Area (%)
1	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	73	2.990	1 015 462	99	234	3.57
2	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) glycolic acid-di-TMS	73	8.513	115 772	97	220	0.41
3	Butanedioic acid, bis(trimethylsilyl) ester (cas) di-TMS succinate	147	8.513	178 105	99	262	0.63
4	Benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate	209	13.628	54 140	90	224	0.90
5	Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]- (cas) monotrimethylsilyl vanillin	194	13.628	48 497	99	224	0.17
6	Benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate	209	22.268	2 110 418	98	308	7.43
7	Benzoic acid, 5-methoxy-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) 5-methoxysalicylic acid-di-TMS	297	22.268	2 258 651	99	312	7.95
8	(3-hydroxy-4-methoxyphenyl)ethylene glycol tris(trimethylsilyl) ether	297	23.914	85 336	99	400	0.30
9	Benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	297	24.897	71 312	98	312	0.25
10	Vanilethanediol 3-TMS	73	25.155	168 080	99	400	400

Figure 4



Gas chromatography mass spectrometry of the active sample of fraction B from the ethanolic extract of *Pericampylus glaucus*.

The high reduction in blood glucose level through the crude ethanolic extract of *P. glaucus* occurs because of the presence of various phytochemicals that are responsible for attenuations in blood glucose levels and synergistic effects against blood glucose levels [14].

This attenuation might be because of potentiation of insulin stimulated in plasma by aggregation of the release of insulin in the pancreas from accessible beta cells or its release from bound insulin, producing the secretion of greater quantities of insulin [15].

The glucose-lowering effect might be because of enhanced insulin release similar to the actions of oral hypoglycemic allopathic drugs such as sulfonylureas [16].

This suggests the existence of alkaloids in the tested crude ethanolic extract that might exert antidiabetic effects, along with other constituents, to have significant effect against high blood glucose level [17]. The antidiabetic action could be because of the potentiation of the insulin effect by its release from the bound form or an increase in the secretion of insulin in the pancreas from β cells [18].

An attempt was made to identify the component responsible for the above-mentioned pharmacological action in the active crude ethanolic extract of *P. glaucus*. The identification of the constituent responsible for hypoglycemic and hypolipidemic activities was performed through the column chromatography technique and GCMS analysis.

The identification of the active fraction was performed using the technique reported by Gayathri and Kannabiran [19]. The active crude ethanolic extract was divided into various fractions using different solvent systems and four fractions were collected (A, B, C, and D) [19].

In STZ-induced diabetic rats, 'fraction B,' which was collected from petroleum ether and ethyl acetate, was found to show considerable ($P<0.001$) antidiabetic activity at a dose of 100 mg/kg body weight compared with the other fractions collected (fractions A, C, and D).

The animals that were treated by different fractions of *P. glaucus* did not show considerable ($P>0.05$) attenuations in blood glucose levels, except for ethanol.

The three fractions (A, C, and D) could not reduce glucose levels considerably, indicating that these fractions lacked constituents with hypoglycemic

activity or that the active constituents also had antagonistic ingredients.

Many researchers have explained that the significant effects of plant fractions against high blood glucose levels are because of the presence of hypoglycemic constituents such as saponins, triterpines, alkaloids, and flavonoids [20,21].

The presence of alkaloids and terpenes in plants is responsible for significant reductions in blood glucose levels because of inactive in renovating the biochemical values induced by STZ. Therefore, the major effect that was found in fraction 'B' of the ethanol extract of *P. glaucus*, but not in the other fractions (A, C, and D), may be responsible for the antidiabetic activity, along with other components as several alkaloids have been reported to lead to significant reductions in high fasting blood glucose levels [22,23].

The active fraction was tested at a dose of 50 mg/kg body weight. The blood glucose level was determined after the active fraction was administered along with the standard drug glibenclamide. A considerable ($P<0.001$) increase in blood glucose was noted in animals that received STZ as matched to normal control group. The experimental rats that received the active fraction at a dose level of 50 mg/kg body weight showed a meaningful attenuation ($P<0.001$) in blood glucose levels.

The active fraction from the leaves of *P. glaucus* (Lam.) Merr and the standard drug glibenclamide induced considerable alterations in serum lipid levels of total cholesterol, triglycerides, LDL, and HDL in experimental STZ-induced diabetic animals.

The active fraction was found to be effective in improving ($P<0.001$) attenuations in the total triglycerides, LDL, and total cholesterol, and increases in HDL in tested animals compared with the diabetic control animals.

Fraction B showed positive hypoglycemic and hypolipidemic effects against high blood glucose levels and lipid profiles in the animals models.

To identify the compounds responsible for antidiabetic and antihyperlipidemic activities associated with 'B,' respectively, a GCMS analysis was carried out. Ten peaks were noted that indicated the presence of 10 compounds: propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester, acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) glycolic acid-di-TMS, butanedioic acid, bis(trimethylsilyl) ester (cas) di-TMS succinate, benzoic acid, 2-[(trimethylsilyl) oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate, benzaldehyde, 3-methoxy-4-[(trimethylsilyl) oxy]-(cas) monotrimethylsilyl vanillin, benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate, benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate, benzoic acid, 5-methoxy-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) 5-methoxysalicylic acid-ditm, (3-hydroxy-4-methoxyphenyl)ethylene glycol tris(trimethylsilyl) ether, benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, and vanilethanediol 3-TMS. Amongst with identified compounds from active fraction no pharmacological activities have been reported on maximum compounds; only three compounds have been documented to have various known pharmacological properties such as free radical scavenging, antiproliferative, anticancer, antifungal, and antimicrobial in general.

In a study, it was reported that the phytochemicals such as propanoic acid, 2-[(trimethylsilyl)oxy]-, and trimethylsilyl ester possess antioxidant and antiproliferative properties [24].

A study reported that benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester, isolated from the stem bark of '*Peltophorum africanum*,' was effective against microorganism and fungal infections and safety effect on normal human liver cell [25]. Therefore, benzoic acid, 3-methoxy-4-[(trimethylsilyl) oxy]-, trimethylsilyl ester, one of the compounds also isolated from the active fraction of *P. glaucus*, might be useful in future for the treatment of bacterial and fungal infections.

The generation of free radicals in the body is linked to the pathogenesis of many chronic diseases such as diabetes, cancer, hypertension, and hyperlipidemia. Many well-known researchers have confirmed the role of antioxidants in the prevention of long-term diseases such as diabetes and cancer. This research has proven the role of propanoic acid, 2-[(trimethylsilyl) oxy]-, trimethylsilyl ester, in scavenging of free radicals in the body. The above GCMS studies also showed the presence of propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester compound in the active fraction of *P. glaucus;* thus, the hypoglycemic and hypolipidemic activities of *P. glaucus* might be because of the presence of propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester [26]. At present, the exact compound isolated from the active fraction responsible for the above activities is unknown. The exact compound and

mechanism of action of compounds from the active fraction will be the subject of further studies.

Conclusion

The present research work reported the use of *P. glaucus* leaves in the ayurvedic system of medicines to treat diabetes in Malaysia. The present research work also identified the presence of compounds in *P. glaucus* leaves responsible for their hypoglycemic action. Further comprehensive research works are needed to determine the exact compounds and elucidate the exact mechanism of the hypoglycemic effect of *P. glaucus* leaves.

Acknowledgements

The authors are grateful to the Faculty of Pharmacy, Lincoln University College, Malaysia, for providing facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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