RESEARCH ARTICLE

Antioxidant and antidiabetic activity of isolated flavonoids from *Alangium salvifolium* leaves extracts

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Abstract

Background: *Alangium salvifolium* is content of flavonoids and mainly used for management of diabetes in India. In the present study, we evaluated the antioxidant and antidiabetic activity of isolated flavonoids from *A. salvifolium* leaves extracts in experimental animals. **Materials and Methods:** The petroleum ether, ethanol and aqueous extracts of leaves of *A. salvifolium* were prepared by successive Soxhlet extraction process, and further phytochemical screening and *in vitro* antioxidant were performed. The most effective ethanol extracts of *A. salvifolium* were selected for separation of flavonoids compound, and consequently qualitative phytochemical analysis was done. The flavonoid containing fractions were screened for antidiabetic activity in streptozotocin (STZ) induced diabetic rats. **Results:** The ethanol and aqueous extracts of *A. salvifolium* contain the maximum number of phytoconstituents such as alkaloids, glycosides, carbohydrates, flavonoids, tannins, and polyphenol. The findings of *in vitro* antioxidant activity confirmed that ethanol extracts expressed higher antioxidant activity compared to aqueous extract, and hence ethanol extracts were further selected for the isolation of various fractions. The FAS6 to FAS7 indicate the presence of phenolic compounds and flavonoids. The FAS6 and FAS7 significantly reduced the blood glucose level, total cholesterol, low-density lipoprotein, triglycerides and a significant rise in high-density lipoprotein in STZ-induced-diabetic rats as compared to the diabetic control group. **Conclusion:** Based on the results of the antidiabetic activity of FAS6 to FAS7 isolated from leaves of *A. salvifolium* were due to its antioxidant property.

Key words: *Alangium salvifolium, antidiabetic activity, antioxidant activity, flavonoids*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, mainly characterized by disruption in carbohydrates, protein, and fat metabolism caused by the complete or relative insufficiency of insulin action. Diabetes mellitus is one of the most common chronic diseases in the whole world. It is a complex, multifactorial disease which affects the quality, quantity, and style of an individual's life.[1-3] The fact confirmed by reports from the World Health Organization (WHO) shows that India has the largest number of diabetic subjects in the world. Hyperglycemia can be handed initially with oral synthetic agent and insulin therapy. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment. In the natural system of medicine, many plants have been claimed to be useful for the treatment of diabetes mellitus. The dependence of large rural population on medicinal plants for treatment of diabetes is because of its availability and affordability. In addition, after the approbation made by the WHO on diabetes mellitus, exploration on hyperglycemic agents from medicinal plants has become more significant. Medicinal plants continue to provide valuable therapeutic agents, both in modern and traditional medicine.[4,5]

Flavonoids are well-known for their multi-directional biological activities including antidiabetic efficacy. Plant containing flavonoids or polyphenol is good choice to use as antidiabetic drugs.[6]*Alangium salvifolium* is the content

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of flavonoids and mainly used as medicine in India, China, and the Philippines. It is the most versatile medicinal plants having a wide spectrum of biological activity. *A. salvifolium* showed potent anticancer, diuretic, anti-inflammatory, antimicrobial, laxative, astringent, emollient, anthelmintic, and antiepileptic activities. The plant was also reported for its antifungal activity, antimicrobial activity, cardiac activity, and antifertility activity.[7,8] In Ayurveda almost all parts of the tree used for medicinal purposes. The roots and the fruits are used for the treatment of rheumatism, leprosy, and hemorrhoid. Externally, it is used for the treatment of bites by rabbits, rats, and dogs. Root bark is an antidote for several poisons. Fruits are sweet, cooling and purgative and used as a poultice for treating burning sensation and hemorrhage.[9]

The antidiabetic activity of *A. salvifolium* leaves extract was scientifically reported. However, no report has yet been published about the active component responsible for antidiabetic activity of *A. salvifolium* leaves extract. We planned to isolate the flavonoids and polyphenol components from *A. salvifolium* leaves extract. The main aim of this study was to evaluate the antioxidant and antidiabetic activity of isolated flavonoids from *A. salvifolium* leaves extracts.

MATERIALS AND METHODS

Plant Material

A. salvifolium of vouchered herbarium specimen was prepared and preserved along with crude drug sample to the Department of Botany, Government P.G. College, BHEL, Bhopal (M.P.), India. The leaves were shade dried, reduced to coarse powder and stored in an airtight container until further use.

Preparation of Extract

The powdered leaves of *A. salvifolium* about 1 kg were packed in Soxhlet apparatus and extracted with petroleum ether, ethanol and distilled water separately, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure to remove the solvent completely, and later dried in a desiccator.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening was performed to identify secondary metabolites (phytoconstituent) in extracts.[10]

In Vitro Antioxidant Activity of Extract

Hydrogen-donating activity

The methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (100 mM and 2.95 ml), 0.05 ml of extracts dissolved in methanol was added at different concentrations (50–250 µg/ml). The reaction mixture was shaken, and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into a percentage of antioxidant activity (%AA). Ascorbic acid was used as a standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation:

$$\% AA = 100–\{[(Abs_{sample}–Abs_{blank})\times 100]/Abs_{DPPH}\}$$

The inhibitory concentration (IC_{50}) parameter of hydroalcoholic and aqueous extracts was determined using Microsoft Excel 2007.

Total polyphenol content

Total polyphenol content was determined using the colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin–Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg/100 ml of the sample (extract).

Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg/100 ml of the sample.

Reducing power assay

The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, and pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL and 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 µg/mL extracts aliquot.[11,12]

Isolation of Compound from Ethanol Extracts

The *A. salvifolium* extract was subjected to column chromatography using silica gel (60–120 mesh size), and eluted with the following solvent ratios of Hexane:ethyl acetate (EA), 100:0, 75:25, 50:50, 25:75, and 0:100, then with 100:0, 75:25, 50:50, 25:75, and 0:100, EA:ethanol (Eth). The fractions (25 ml) were collected from the column. The elute collected was monitored by thin

layer chromatography (eluent: EA-Eth, 9:1 and 3:2) for homogeneity and the similar fraction was pooled together. The eight different fractions were collected and dried (Table 5.3). The fractions FAS1, FAS2, and FAS3 were containing waxy material; the fractions FAS5 and FBP8 were powder, but quantity was very little. The yield of fractions FAS4, FAS6, and FAS7 was 180 mg, 410 mg, and 305 mg, respectively. The three fractions were further analyzed for phytochemical screening to determine the nature of the isolated compound.[13]

Pharmacological Activity

Selection of animals

Male Wistar rats (150–200 g) were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3°C, relative humidity 65 ± 10%) for 12 h in the dark and light cycle, respectively, and were given standard food and water *ad libitum*. All experiments were approved by the Institutional Ethical Committee and were carried out according to the Animal Ethics Committee guidelines.

Oral glucose tolerance test (OGTT) of A. salvifolium fractions

The OGTT[8] was performed in overnight fasted (18 h) normal rats. The rats were divided into seven groups ($n = 6$). Group I served as normal control rats, administered drinking water daily; Group II had glucose control rats; Group III rats were administered standard drug Glibenclamide (0.5 mg/kg); Group IV rats were administered FAS6 (25 mg/kg); Group V rats were administered FAS (50 mg/kg); Group VI rats were administered FAS7 (25 mg/kg); and Group VII rats were administered FAS7 (50 mg/kg). Glucose (2 g/kg) was fed to rats of Group II–Group VII, 30 min before the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 min of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase-peroxidase glucose estimation kit.[14,15]

Induction of non-insulin dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted adult Wistar strain albino male rats weighing 170–220 g by a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ), 15 min after i.p. administration of 120 mg/kg of nicotinamide. STZ was dissolved in a citrate buffer (pH 4.5), and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study.[16,17]

Experimental design

Evaluation of antidiabetic activity of A. salvifolium fractions

The animals were segregated into seven groups of six rats each. The extract was administered for 28 days. Group I served as normal control rats, administered drinking water daily for 28 days; Group II had diabetic control rats, administered drinking water daily for 28 days; Group III diabetic rats were administered standard drug Glibenclamide (0.5 mg/kg); Group IV rats were administered FAS6 (25 mg/kg); Group V rats were administered FAS (50 mg/kg); Group VI rats were administered FAS7 (25 mg/kg); and Group VII rats were administered FAS7 (50 mg/kg) for 28 days. The fasting glucose levels were determined on 0, 7th, 14th, and 28th day of extract administration. During the experimental period, the rats were weighed daily, and the mean change in body weight was calculated.

Estimation of biochemical parameters

The biochemical parameters were determined on day 12 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined by the glucose oxidase method, using an auto-analyzer.[18-21]

Data analysis

Results were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey's test using statistical software package, GraphPad Prism; version 5.03. Values were expressed as a mean ± standard error of the mean and $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical Screening of *A. salvifolium* Extract

Preliminary phytochemical investigations of the extracts of leaves of *A. salvifolium* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats, and carbohydrates. The flavonoids and polyphenol along with other phytoconstituents present in ethanol and aqueous extract and details are presented in Table 1. The maximum phytoconstituents were observed in ethanol extracts of leaves of *A. salvifolium* [Table 1].

In Vitro Antioxidant Activity

The ethanol and aqueous extracts of *A. salvifolium* were subjected to *in vitro* antioxidant studies to determine and compare the antioxidant activities of extracts.

Hydrogen-donating activity of A. salvifolium

As shown in Table 2 and 6.4, *A. salvifolium* of ethanol and aqueous extracts strongly scavenged DPPH radical

Table 1: Phytochemicals present in leaves of *A. salvifolium* extracts

Phytoconstituent	Pet. Ether	Ethanol	Aqueous
Alkaloids			
Dragendorff's test	–	+	–
Hager's test	–	–	–
Mayers	–	+	–
Wagners	–	–	–
Glycosides			
Legal's test	–	+	+
Keller–Killiani test	–	–	+
Baljet test	–	–	–
Keller–Killiani test	–	+	+
Borntrager's test			
Carbohydrates			
Molish test	–	+	+
Benedict's test	–	–	+
Fehling's test	–	+	+
Tannins and Phenolic compound			
5% $Fecl_3$ solution	–	+	+
Lead acetate solution	–	+	+
Bromine water	–	–	–
Potassium ferric cyanide and ammonia solution			
Flavonoids	–	–	–
Shinoda test	–	+	+
Steroid test			
Liebermann–Burchard test	–	–	–
Salkowski test	–	–	–
Protein			
Salkowski test	–	–	–
Biuret test	–	–	–
Ninhydrin test			
Fat and oil test			
Saponification test	–	–	–
Spot test	+	–	–

+: Present, –: Absent. *A. salvifolium: Alangium salvifolium*

with the IC_{50} being 130.79 and 211.01 µg/ml, respectively [Figures 1 and 2]. The scavenging was found to dose-dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 96.04.

Total phenolic content of A. salvifolium

The ethanol and aqueous extract of *A. salvifolium* was evaluated for investigation of the total phenolic content concentrations in extracts. Standard curve of gallic acid was prepared in distilled, and the linear equation of gallic acid was found to be y = 0.0373 x + 0.0125 [Figure 3]. The results of the total phenolic content of the extracts examined, using Folin–Ciocalteu method, are depicted in Table 2. The total phenolic content of ethanol and aqueous extract of *A. salvifolium* were 74.21 and 56.48 GAE mg/g, respectively.

Total flavonol content of A. salvifolium

The concentrations of flavonoids in ethanol and aqueous extract of *A. salvifolium* were determined spectrophotometrically using aluminum chloride. The content of flavonoids was expressed in terms of QEs. Standard curve of quercetin was prepared in distilled, and the linear equation of quercetin was found to be y = 0.0272 x +0.0164 [Figure 4]. The content of flavonoids identified in the tested extracts is shown in Table 3. The concentrations of flavonoids in ethanol and aqueous extract of *A. salvifolium* were 74.31 and 59.26 QE mg/gm, respectively.

Table 2: Free radical scavenging capacity of ethanol and aqueous extracts of *A. salvifolium*

Concentration (µg/ml)	DPPH scavenging %		
	Ethanol extract	Aqueous extract	Ascorbic acid
50	26.63±0.28	11.25±0.58	96.04±0.59
100	41.38±0.47	18.34±0.93	-
150	56.72±0.63	30.48±0.18	-
200	69.51±0.49	46.17±0.32	-
250	82.69±0.92	63.29±0.78	-
IC_{50}	130.79	211.01	-

Values are mean±SEM of six determinations. *A. salvifolium: Alangium salvifolium,* IC_{50}: Inhibitory concentration, DPPH: 2,2-diphenyl-1-picrylhydrazyl. SEM: Standard error of the mean

Table 3: Determination of total polyphenol and flavonol content of *A. salvifolium*

Extract	Total polyphenol content (GAE mg/gm)	Total flavonol content (QE mg/gm)
Ethanol	74.21±0.86	74.31±0.92
Aqueous	56.48±0.42	59.26±0.38

Values are mean±SEM of triplicate determinations.
A. salvifolium: Alangium salvifolium, QE: Quercetin equivalent, SEM: Standard error of the mean

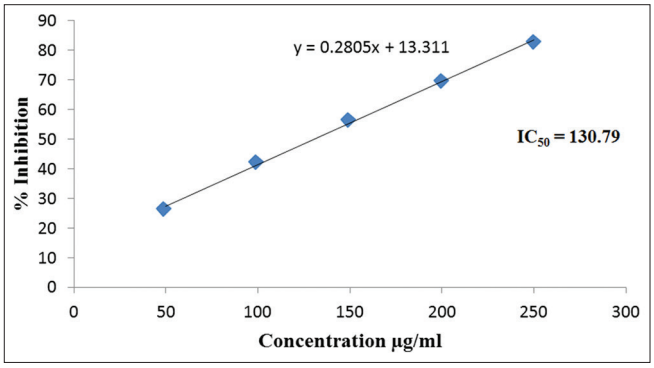

Figure 1: Inhibitory concentration values of ethanol extracts of *Alangium salvifolium*

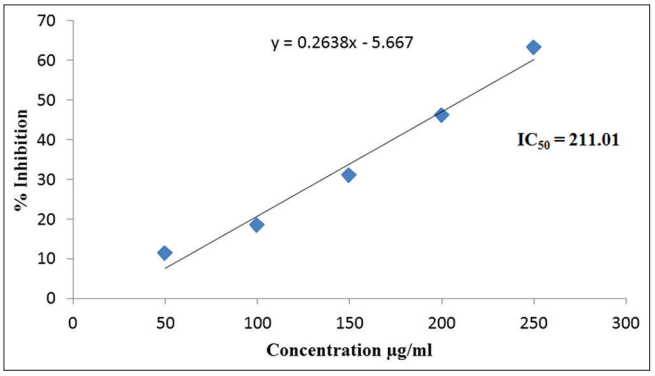

Figure 2: Inhibitory concentration values of aqueous extracts of *Alangium salvifolium*

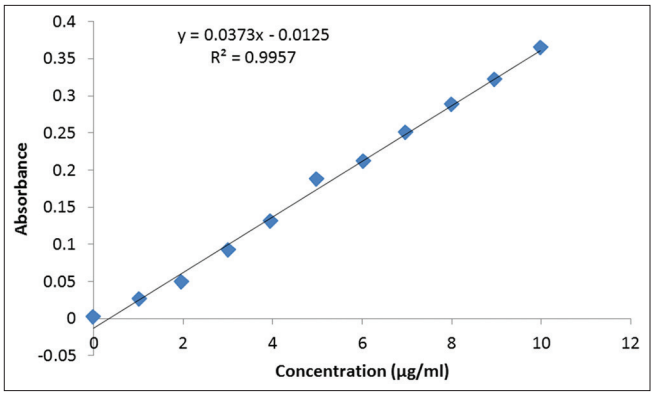

Figure 3: Calibration curve of gallic acid in distilled water

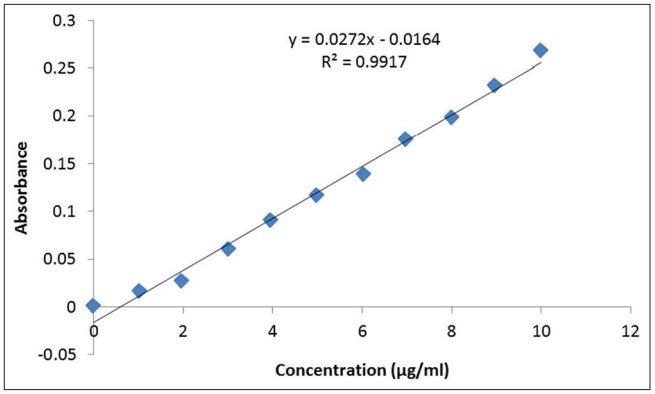

Figure 4: Calibration curve of quercetin in distilled water

Reducing power assay of A. salvifolium

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 4 revealed that the antioxidant activity of ethanol and aqueous extract of *A. salvifolium*. The reducing power of the ethanol and aqueous extract of *A. salvifolium* were found to be 44.58% and 27.46%, respectively.

Isolation of Compound from Ethanol Extract of *A. salvifolium*

The ethanol extract of *A. salvifolium* leaves was subjected to column chromatography, and fractions were eluted with the gradient polarity of solvent, namely hexane, EA, and ethanol. The eight different fractions were collected and dried. The fractions FAS1, FAS2, and FAS3 were containing waxy material; the fractions FAS5 and FBP8 were powder, but quantity was very little. The yield of fraction FAS4, FAS6, and FAS7 was 180 mg, 410 mg, and 305 mg, respectively. The three fractions were further analyzed for phytochemical screening to determine the nature of the isolated compound.

Preliminary Phytochemical Analysis of Isolated Fraction of Ethanol Extract of *A. salvifolium*

The phytochemical investigation of FAS4 of *A. salvifolium* leaves revealed the presence of alkaloids, glycosides, and carbohydrates. The FAS6 and FAS7 indicate the presence of tannins and phenolic compounds and flavonoids [Table 5].

Antidiabetic Activity of Isolated Fractions

The *in vitro* studies of the FAS6 and FAS7 isolated from *A. salvifolium* leaves extracts indicate the presence of flavonoids and polyphenol. The antidiabetic activity on FAS6 and FAS7 was performed.

Table 4: Antioxidant activity determination of *A. salvifolium*

Particulars	Absorbance at 700 nm	Antioxidant activity (%)
Ascorbic acid	0.812±0.03	100.00
Ethanol extract	0.362±0.02	44.58
Aqueous extract	0.223±0.04	27.46

Values are mean±SEM of triplicate determinations.
SEM: Standard error of the mean, *A. salvifolium: Alangium salvifolium*

Table 5: Preliminary phytochemical analysis of isolated fraction of ethanol extract of *A. salvifolium*

Phytoconstituents	FAS4	FAS6	FAS7
Alkaloids	+	−	−
Glycosides	+	−	−
Carbohydrates	+	−	−
Tannins and phenolic compounds	−	+	+
Flavonoids	−	+	+
Steroids	−	−	−
Proteins and amino acids	−	−	−
Fixed oils and fats	−	−	−

(+) Present, (−) Absent. *A. salvifolium: Alangium salvifolium,*
SEM: Standard error of the mean

OGT effects of A. salvifolium fractions

The effects of fractions of FAS6 and FAS7 of *A. salvifolium* on the plasma glucose level are shown in Table 6. After administration of glucose in rats, the rise in glucose level was observed in glucose control, extract treated, and standard group. In rats treated with fractions FAS6 and FAS7, there was a significant reduction in plasma glucose level, while in glucose control rats the plasma glucose level increased. Meanwhile, same results were observed in glibenclamide treated group. The fraction FAS7 exhibited maximum decreased in plasma glucose level compared with other fractions.

Effect on NIDDM of A. salvifolium fraction

Induction of diabetes in experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of a fraction of *A. salvifolium*, on serum glucose levels of normal, and STZ -induced rats, is shown in Table 7.

The animals treated with STZ, namely Group II, a significant increase in serum glucose level was observed on 0, 7th, 14th, and 28th day when compared with normal group rats (Group I). The Group III received glibenclamide (0.5 mg/kg p.o.) showed a significant decrease in serum glucose level when compared with diabetic control rats. After the oral administration of fraction in diabetic control rats, a significant reduction in blood glucose level was observed when compared with diabetic control rats. Moreover, the administration of fractions in diabetic control rats, also significantly decreased the serum glucose level compared with diabetic control rats. The outcomes exhibited that FAS6 and FAS7 treated animal showed significantly decreased in the blood glucose level of diabetic rats on the 7th day. From results, it has been observed that the FAS7 showed maximum activity as compared to other fractions.

Antihyperlipidemic activity of A. salvifolium fractions

The outcomes of lipid profiles in control and experimental rats are exhibited in Table 8. The rats of diabetic control showed a significant increase in serum TGL, total cholesterol, and LDL while the increase in HDL when compared with normal. The rat treated with glibenclamide also reduced TGL, total cholesterol, LDL, and increased HDL when compared with diabetic control group. The fraction FAS6 and FAS7 showed a significant decrease in total cholesterol, LDL, and TGL and a significant increase in HDL when compared with diabetic control group. All these effects were observed on day 28th. From the result of lipid profile, it has been observed that the FAS7 exhibited maximum antihyperlipidemic activity compared with other fractions.

Table 6: Effect of fractions of *A. salvifolium* on OGTT

Group	Plasma glucose concentration (mg/dl)		
	0 min	30 min	90 min
Normal Control	75.24±4.21	78.15±3.46	77.32±1.89
Glucose control	74.81±2.53	192.24±5.67[a]	143.61±2.18[a]
Glucose+Glibenclamide (0.5 mg/kg)	78.43±4.26	112.91±4.76*	75.13±4.17*
FAS6 (25 mg/kg)	79.52±5.62	160.14±2.42	98.42±3.16*
FAS6 (50 mg/kg)	76.82±4.38	122.36±5.39*	82.73±4.28*
FAS7 (25 mg/kg)	74.28±3.82	143.24±2.83	92.26±1.85*
FAS7 (50 mg/kg)	79.15±6.14	116.73±4.18*	76.31±1.24*

Values are expressed as mean±SEM (number of animals, *n*=6); significantly different at [a]*P*<0.05 when compared with normal control group, **P*<0.05 when compared with diabetic control group. *A. salvifolium: Alangium salvifolium,* SEM: Standard error of the mean, OGTT: Oral glucose tolerance test

Table 7: Effect of fractions of *A. salvifolium* on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal control	76.14±6.42	79.32±2.85	75.83±5.28	76.36±7.18
Diabetic control (STZ)	139.43±3.72[a]	186.49±4.94 [a]	231.72±6.31 [a]	264.53±4.92[a]
Diabetic+standard Glibenclamide (0.50 mg/kg)	145.28±4.53	116.65±6.28*	88.72±4.57*	73.41±4.12*
FAS6 (25 mg/kg)	134.36±2.49	153.72±4.58	124.18±6.43*	95.14±5.39*
FAS6 (50 mg/kg)	143.63±5.18	129.42±6.41*	101.78±3.19*	81.35±6.42*
FAS7 (25 mg/kg)	140.92±6.79	142.48±5.68	118.39±4.27*	85.68±4.25*
FAS7 (50 mg/kg)	135.17±5.43	117.83±4.19*	92.86±4.36*	76.15±5.73*

Values are expressed as mean±SEM (number of animals, *n*=6); significantly different at [a]*P*<0.05 when compared with normal control group, **P*<0.05 when compared with diabetic control group. STZ: Streptozotocin, SEM: Standard error of the mean, *A. salvifolium: Alangium salvifolium*

Table 8: Determination of biochemical parameters after treatment with fractions of *A. salvifolium*

Group	Lipid profile (mg/dl)			
	TGL	Total cholesterol	HDL	LDL
Normal control	81.72±2.57	89.23±4.18	63.58±3.59	51.36±5.86
Diabetic control (STZ)	189.43±4.32[a]	167.24±2.36 [a]	24.19±4.32[a]	149.75±2.73[a]
Diabetic+standard Glibenclamide (0.50 mg/kg)	80.73±6.14*	83.68±4.72*	60.73±5.76*	56.92±4.38*
FAS6 (25 mg/kg)	136.83±3.47	124.38±5.39*	30.62±3.83	121.76±6.53
FAS6 (50 mg/kg)	90.79±3.65*	95.14±7.18*	58.95±2.59*	63.29±4.24*
FAS7 (25 mg/kg)	129.68±4.32	120.39±5.64*	35.17±4.49	117.81±5.17
FAS7 (50 mg/kg)	82.43±5.24*	85.16±3.47*	64.28±5.93*	56.57±3.48*

Values are expressed as mean±SEM (number of animals, *n*=6); significantly different at [a]*P*<0.05 when compared with normal control group, **P*<0.05 when compared with diabetic control group. SEM: Standard error of the mean, TGL: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, *A. salvifolium: Alangium salvifolium,* STZ: Streptozotocin

DISCUSSIONS

In the recent days, many researchers and investigators tested various traditional medicinal plants for their potential antidiabetic effect in experimental animals. Working on the same line, *A. salvifolium* was selected for isolation of active constituents from extract and evaluate antidiabetic activity of isolated compounds.

Preliminary phytochemical investigations of the ethanol and aqueous extracts of leaves of *A. salvifolium* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, and carbohydrates. Now, ethanol and aqueous extracts of *A. salvifolium* were selected for further *in vitro* antioxidant activity evaluation as this extract revealed the presence of flavonoids and phenolic compounds.

The ethanol extracts exhibited the highest amount of total polyphenol and flavonol content compared to aqueous extracts. It is well documented that plant flavonoids and phenols, in general, are greatly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used for the prevention and cure of various diseases, which are mainly associated with free radicals. The findings of total polyphenol and flavonol content of ethanol and aqueous extract of *A. salvifolium* supported the study of DPPH scavenging capacity of extracts.

The reducing power of ascorbic acid was found to be higher than ethanol and aqueous extracts. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability. The ethanol extract of *A. salvifolium* might, therefore, contain a high amount of reductions than aqueous extract. The result indicates that extract act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. During the study, it was found that antioxidant activity was produced due to the presence of phenolic compounds. So here reducing power assay justify that ethanol extract of *A. salvifolium* contains the maximum amount of the total polyphenol and flavonol.

From the results of antioxidant, it can be concluded that ethanol extracts of *A. salvifolium* produce higher antioxidant activity compared to aqueous extract and could alleviate the number of oxidative stress-induced diabetes. The above study was done only for the proper selection of extracts from *A. salvifolium*, to isolate the active constituent from extract expressing maximum antioxidant activity. Hence, the ethanol extract of *A. salvifolium* was selected for the isolation of active constituents by column chromatography.

The fractions obtained from the ethanol extract of *A. salvifolium* exhibited various types of secondary metabolites. Many investigations have proven that varieties of flavonoid molecules possess antidiabetic activity. Thus, it may be valuable to continuously evaluate the antidiabetic activity of flavonoids, not only for establishing antidiabetic mechanisms but also for developing a new class of antidiabetic agents. The FAS6 and FAS7 containing polyphenol and flavonoids compound and these organic substances impart chief role in the antidiabetic activity. Hence, this result supports us to evaluate the antidiabetic activity of the FAS6 and FAS7.

STZ, a monofunctional nitrosourea derivative, derives diabetogenic activity due to its ability to induce oxidative stress and damage in β-cells. STZ can selectively attack pancreatic β-cells by producing free radicals of oxygen, nitrogen monoxide, and reducing intracellular nicotinamide adenine dinucleotide (NAD), and NAD phosphate (NADP), which are crucial for the electron delivery and energy metabolism in β-cells.[7,20]

The diabetes was induced on rats after administration of STZ. The fractions of *A. salvifolium* were screened for STZ -induced antidiabetic activity. The FAS6 and FAS7 significantly reduced the blood glucose level in STZ-induced-diabetic rats as compared to the diabetic control group. Moreover, the FAS6 and FAS7 increased the body weight of diabetic rats. The possible mechanism by which *A. salvifolium* brings about its hypoglycemic action in the diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

In general, it has been observed that hyperlipidemia is a complication associated with hyperglycemia. During the study, it was observed an increase in total cholesterol, TGL, LDL, and decrease in HDL in STZ induced diabetic rats as compared to normal animals. The FAS6 and FAS7 showed a significant reduction in total cholesterol, LDL, and TGL and a significant rise in HDL when compared with diabetic control group. The potent antidiabetic effect of the plant extract suggests the presence of potent antidiabetic active principles, which produced an antihyperglycemic effect in diabetic rats. The outcomes of lipid profile confirmed the potent antidiabetic activity of FAS6 and FAS7.

CONCLUSION

The FAS6 to FAS7 of *A. salvifolium* exhibited strong antioxidant activity and may confer a beneficial effect against oxidative stress. Based on the results of *in vivo* assay, the antidiabetic activity of FAS6 to FAS7 isolated from leaves of *A. salvifolium* was due to its antioxidant property. From findings, it has been concluded that the FAS7 of *A. salvifolium* exhibited maximum antidiabetic activity compared to FAS6.

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