ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITIES OF LEAVES EXTRACTS OF *ALANGIUM SOLVIFOLIUM* (LINN.F) WANG.

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Summary

A preliminary laboratory trail was performed to evaluate the presence of various phytoconstituents, anti-diabetic and anti-oxidant activities of chloroform and methanolic extract of leaves of *Alangium solvifoium* (Linn.f) Wang. The anti-diabetic activity was performed for both chloroform and methanolic extract through oral route of administration for 15 days on alloxan induced hyperglycemic rats and the activity was compared with the standard drug Glibenclamide. The anti-oxidant activity was also performed for both the extracts by DPPH (1, 1-Diphenyl-2-picryl hydrazide) radical scavenging method. Reduction of blood glucose, total cholesterol and increase in total protein levels were used to measure the anti-diabetic activity. The decrease in absorbance of methanolic solution of DPPH was the measure of antioxidant activity. From this evaluation the methanolic extracts of leaves of *A.solvifolium* exhibited better anti-diabetic and anti-oxidant activity than the chloroform extract.

Keywords: *Alangium solvifolium*, Anti-diabetic, Anti-oxidant, Alloxan, Glibenclamide, DPPH (1,1-Diphenyl-2-picryl hydrazide).

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Introduction

Diabetic mellitus (DM) is one of the most common endocrine disorder that affects more than 100 million people world wide (6% of the population) and by the year 2025 it is expected to affect five times the number of people that is affecting today(1, 2). More than 400 plants are known to have been recommended and recent investigations have affirmed the potential value of some of these treatments. Chemical studies directed at the isolation, purification and identification of the substances responsible for the anti-diabetic activity also being conducted (3). In recent years, interest on herbal agents as therapeutic treatment option has increased due to their limited side effects. Most of these products have been found to be non toxic in animal studies done so far. Presently, there are some isolated reports where attempts have been made to understand the mode of hypoglycemic activities of some folk medicines in India (4, 5).

The oxidation induced by oxygen-centered free radicals can result in cell membrane disintegration, membrane protein damage, DNA mutation and oxidative damage of lipids. They play an important role in oxidative stress related to the pathogenesis of various diseases. Many anti-oxidant compounds, both synthetic and natural, have been identified as free radical or active oxygen scavengers. In recent years, there has been a global trend towards the use of natural phytochemical present in natural resources, such as spices, herbs, tea, enzymes, protein and protein hydrolysates. Herbs have been utilized since antiquity for their culinary qualities and in addition, have also been used for their preservative and medicinal properties (6).

The plant *A.solvifolium* is commonly known as sage leaved Alangium in English. In Sanskrit and Tamil known as Anokata and Alangi respectively. In traditional system of medicine the various parts of plant were used as anti-leprosy, anti-inflammatory, anti-diabetic and anti-rheumatic agent. It is also used as anti-dotes for several poisons (7-9).

In the light of the above information the present investigation was under taken to evaluate the glucose lowering effects and free radical scavenging effects of chloroform and methonolic extracts of *A.solvifolium* in alloxan induced hyperglycemic rats to establish pharmacological evidence in support of folkloric claim.

Materials and methods

Plant material: The leaves of *A. solvifolium* were collected in and around the college of Pharmacy, SRM University, Kancheepuram District, Tamil Nadu, India during the month of March 2008. The plant was authenticated by Prof. P. Jayaraman**.** Director, PARC (Plant Anatomy Research Center), Chennai, where a voucher specimen (No: PARC/SRMU/08/298) was deposited for future reference. The leaves of *A. solvifolium* was washed with distilled water, shade dried, powdered and stored in an airtight container for further use.

Extraction: 100 gm of coarsely powdered dried leaves of *A. solvifolium* was mixed with 500 ml of chloroform and allowed to stand for 48 hours with occasional shaking. It was then filtered and distilled under vacuum to get a concentrated chloroform extract (CE).

To the marc methanol (500ml) was added and allowed to stand for 48 hours with occasional shaking. It was then filtered and distilled under vacuum to get a concentrated methanolic extract (ME). The extracts were stored under vacuum desiccators for further phytochemical analysis and pharmacological screening.

Phytochemical Screening: A preliminary phytochemical screenings of chloroform and methanolic extract of *A.solvifolium* was carried out as described by Khandewal K.L (10) to find out the presence of various phytoconstituents.

Animals: Male albino rats (Wister strain, weighing 180-200gm) were purchased from Tamil Nadu Veterinary Animal Science University, Madhavaram, Chennai and housed under standard laboratory condition (30^0C ± 2^0C, 60-70% relative humidity and 12 hrs/12hrs day light cycle) and allowed standard pellet rat feed and water *ad libitum*. The animal experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee (IAEC/SRMU/11/2008), SRM College of Pharmacy, SRM University, Kattankulathur.

Toxicity study: An acute toxicity study relating to the determination of LD_{50} value was performed using different doses of the extracts according to the method described by Ghosh *et al* (11).

Experimental design

Anti-diabetic activity: Diabetes was induced experimentally in rats by a single intra peritoneal injection of freshly prepared solution of alloxan monohydrate in normal saline at a dose of 100mg/kg body weight. After 72 hours, blood was collected with aseptic precaution from the tail vein of rats under light ether anesthesia and blood glucose levels were determined using Auto-analyzer Micro Lab 2000 (Hamilton, UK). The animals were considered to be diabetic if the blood glucose levels were above 250mg/dl and those animals were alone used for study. The animals were divided into seven groups of six animals each and are treated as follows.

Groups	Treatment
Group I	Normal control given normal saline only.
Group II	Diabetic control given i.p injection of Alloxan monohydrate.
Group III	Diabetic rats given aqueous solution of Glibenclamide10mg/kg, body weight per day for 15 days.
Group IV	Diabetic rats given suspension of CE 100mg/kg, body weight per day for 15 days.
Group V	Diabetic rats given suspension of CE 200mg/kg, body weight per day for 15 days.
Group VI	Diabetic rats given suspension of ME 100mg/kg, body weight per day for 15 days.
Group VII	Diabetic rats given suspension of ME 200mg/kg, body weight per day for 15 days.

Blood glucose levels were estimated by GOD/POD method. Estimation of total cholesterol was done by Parekh and Jung method (12) and serum total protein was estimated by Lowry *et al* method (13).

Anti-oxidant activity by DPPH radical scavenging method: The hydrogen atoms or electron donation ability of chloroform and methanolic extracts were measured from the bleaching of purple colored methanolic solution of DPPH (14-16). This spectrophotometric method uses stable radical 1, 1-Diphenyl picryl hydrazide (DPPH) as a reagent. 7.886 mg of DPPH was weighed accurately and dissolved in 100 ml methanol to obtain 200µM solution of DPPH. All the sample solution were prepared against two concentration i,e., 0.5 mg/ml and 1.0 mg/ml. To 2 ml of methanolic solution of DPPH, 2 ml of sample solution was added and the mixture was incubated in dark at room temperature for 30 minutes. The degree of free radical scavenging activity in the presence of different concentration of extracts and their absorbance were measured colorimetrically at 517nm.

The degree of free radical scavenging activity was expressed as,

$$\% \text{ inhibition} = \frac{\text{A control --------A sample}}{\text{A control}}$$

Where, A control is absorbance of DPPH alone.

A sample is absorbance of DPPH with different concentration of extracts.

Statistical analysis: Statistical analysis was performed using SPSS software package version 9.05 (SPSS Inc., USA).The values were analyzed by one-way analysis of co-variance (ANOVA) followed by Dunnet's multiple comparison tests (DMCT). All the results were expressed as mean ± SEM for 6 rats in each group and $P<0.001$ was considered as significant.

Results

Phytochemical screening: The preliminary phytochemical studies of CE and ME of *A. solvifolium* contains the presence of alkaloids, glycosides, sterols and flavonoids.

Toxicity study: From the toxicity study it was observed that both the CE and ME is non toxic up to the dose of 5.0g/kg bodyweight and was used in different doses for further studies.

Anti-diabetic activity: Diabetic is a chronic metabolic disorder affecting a major population world wide. A sustained reduction in hyperglycemia will decrease the risk of developing micro- vascular complication (2). The conventional therapies for diabetes have many shortcomings like unwanted side effects and high rate of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy without side effects like that of conventional drugs. The present investigation reports the anti-diabetic effects of CE and ME of leaves of *A.solvifolium* on alloxan induced diabetic rats. Alloxan monohydrate is a compound used in medicinal research to produce animal model for

diabetes mellitus. Thus alloxan injection results in diabetes mellitus due to the destruction of β-cells of islets of langerghans as proposed by many researchers (17, 18). This effect is evident by high level of glucose in animals. The data presented here could provide a base for understanding the exact molecular mechanism of action of these plant active principles.

The data shown in the Table:1 clearly indicates that the diabetic rats treated with CE and ME of *A.solvifolium* for 15 days regained their blood glucose levels very close to the normal level when compared to diabetic control ($p<0.001$). This effect could be attributed to the potentiating insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells or its release from bound insulin (19).

The level of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the high risk of coronary heart diseases (20). The administration of CE and ME extract of leaves of *A.solvifolium* significantly decreases the serum total cholesterol and increases the serum total protein levels in alloxan induced diabetic rats when compared to the diabetic control ($p<0.001$). With respect to the cholesterol lowering property of this leaf extract, it could be suggested that the constituents of the plant extracts, may act as inhibitors for enzymes such as HMG-CoA reductase, which participates in *de novo* cholesterol biosynthesis as has been suggested for some other plants earlier (21).

Table1: Effect of *A. solvifolium* on serum glucose, total cholesterol and total protein in normal and alloxan induced diabetic rats on 15 days treatment.

Group	Treatment	Blood glucose (mg/dl)	Total cholesterol (mg/dl)	Total protein (gm/dl)
I	Normal rats.	110.35±4.03	84.33±2.76	8.21±0.19
II	Diabetic rats.	360.32±19.04	192.42±5.16	6.49±0.38
III	Diabetic+Glibeniclamide	152.11±15.28	96.16±3.46	6.79±0.24
IV	Diabetic +CE (100mg/kg)	215.66±13.5	136.66±3.24	6.94±0.10
V	Diabetic +CE (200mg/kg)	174.17±6.02	120.00±4.43	7.25±0.06
VI	Diabetic +ME (100mg/kg)	219.58±9.47	88.36±4.98	7.07±0.13
VII	Diabetic +ME (200mg/kg)	182.26±5.82	80.72±2.34	7.99±0.07

Values are given as mean ± SEM for six rats in each group. Test group compared with diabetic group. Diabetic group compared with normal control group. $P< 0.001$ was considered as significant.

***In vitro* anti oxidant activity:** An *in vitro* anti-oxidant activity of CE and ME of *A.solvifolium* have been done to substantiate the results of anti-diabetic activity by DPPH radical scavenging method. The oxidative stresses in diabetes also includes shifts in redox balance resulting from altered carbohydrates and lipid metabolism, increase the generation of reactive oxygen species (ROS) by glycation and lipid oxidation and decrease the anti-oxidant defenses. Hypoglycemia induced vascular damage have already been reported. The cellular anti oxidant scavenger have been shown to be depleted under elevated oxidative stress mediated tissue damage by a series of chemical reaction (22). The anti-oxidant activity posses by the two different extract of *A.solvifolium* in two different concentration have been shown in the Table:2.

Table.2. The percentage of anti-oxidant activity of *A. solvifolium* by DPPH method

S.No	Sample	% of anti-oxidant activity	
		0.5 mg/ml	1 mg/ml
1.	Chloroform Extract	22.45	52.12
2.	Methanol Extract	35.20	67.50
3.	Standard (α-tocopherol)	43.52	88.40

Our complete observation from this studies, it may be concluded from the results of that the oral administration of CE and ME of *A. solvifolium* leaves (100 and 200 mg/kg, body weight/day) on 15 days treatment is beneficial in controlling the blood glucose and restores the altered total cholesterols and serum total protein levels in alloxan induced experimental diabetic rats. However on comparisons between the two tests extract (CE and ME) methanolic extracts exhibited maximum activity than that of chloroform extract on both anti-diabetic and *in vitro* anti-oxidant activities. Further pharmacological and biochemical investigation are underway to find out the active constituents responsible for the anti-diabetic and anti-oxidant activities to elucidate it's mechanism of action.

Discussion

From the above study it could be conceived that the CE and ME of leaves of *A.solvifolium* may contain some biomolecules that may sensitize the insulin receptor to insulin or stimulate the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the reestablishment of normal blood glucose level. To understand the exact molecular mechanism of action of this plant extract, study is in progress to identify, isolate and purify the bioactive molecules from this plant extract. In conclusion, the data obtained from the present study indicates that the *A.solvifolium* leaf extract could have some bioactive molecules, which may have beneficial effects as anti-hyperglycemic and free radical scavenging agents. The exact mechanism of action needs further investigation. However the present study gives some preliminary idea that the *A.solvifolium* leaf extract has the potential to act at

multiple sites. Toxicity data have proven that. The dose used in their investigation is far below the LD_{50} of the extract and did not show any toxicity. Further studies on possible usefulness of *A.solvifolium* leaf extract in the treatment of diabetes mellitus are encouraged.

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