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Phytochemical and Hypoglycaemic Evaluation of *Alangium Salvifolium* Root Extract

Ahad H. A[1*], Padmaja B. S[1], Yesupadam P[2], Guruprakash P[1], Sravanthi M[1], and Ramyasree P[1]

[1]Department of Pharmaceutics, College of Pharmacy, Sri Krishnadevaraya University, Anantapur-515003, Andhra Pradesh, India

[2]Department of Pharmacognosy, Balaji College of Pharmacy, Anantapur- 515003, Andhra Pradesh, India

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Abstract

The main purpose of present study was to perform phytochemical screening and explore the anti-hyperglycaemic properties of *Alangium salvifolium* root extract in normal and alloxan induced diabetic rats. *A. salvifolium* root gave maximum extractive values of 6.4 % w/w with Ethanol and other parameters were within limits. The extract gave positive tests for phytosterols, triterpenes, flavonoids, carbohydrates and alkaloids. The ethanolic extract of *A. salvifolium* was found to be nearly as potent as tolbutamide in decreasing the blood glucose levels in normal fasting rats. In normal control group the percent reduction in blood glucose indicated that the extract is fast acting. The extract showed significant decrease in blood glucose, when compared with tolbutamide. The evaluated blood glucose levels in alloxan induced diabetic rats were significantly decreased up to 24th h compared to tolbutamide. A single oral administration of alcoholic extract at doses 100, 250 and 500 mg per kg produced a significant blood glucose reduction in a dose dependent manner in normal and diabetic rats. These data confirm the hypoglycaemic and anti-hyperglycaemic effect of alcoholic extract of *A. salvifolium* root in normal and diabetic rats respectively when compared with standard drug tolbutamide.

Keywords: *Alangium salvifolium*; Ethanolic extract; Alloxan induced; Hypoglycaemic action.

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1. Introduction

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality [1]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [2].

[*] *Corresponding author*: abdulhindustan@rediffmail.com

The family Alangiaceae consists of twenty-two species out of which *Alangium salvifolium* (*Linn*) Wang is mainly used as medicine in India, China and Phillipines. Different parts of this plant are reported to possess acrid, astringent, emollient, anthelmintic, diuretic and purgative properties. It is also used externally in acute case of rheumatism and leprosy [3]. The leaf juice can be applied externally and taken internally in case of rabid dog bite. Root bark is an antidote for several poisons. Fruits are sweet, cooling and purgative and used as a poultice for treating burning sensation and haemorrhage [4]. The leaves are used as a poultice in rheumatism [5]. However, there were not enough scientific investigations on the hypoglycaemic activities conferred to these plants.

2. Materials and Methods

2.1. *Collection and identification of plant material*

The entire plants and roots of *A. salvifolium* were collected from Visakhapatnam, Andhra Pradesh, South India. Prof. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam, identified the plants.

2.2. *Materials*

Alloxan monohydrate was purchased from Loba chemicals (Mumbai, India). Tolbutamide (Hoechst India, Bombay), sodium CMC (SD Fine Chemicals, India), Blood Glucose kit (Dr. Reddy's Laboratories Ltd). Methanol, 95% Ethanol were procured from SD fine chemicals Mumbai, India. All the chemicals used were of AR grade and deionized water was used throughout the experiment.

2.3. *Preparation of ethanolic extracts*

Shade dried powdered material of *A. salvifolium Linn* roots were extracted in soxhlet extraction apparatus. The extracts were concentrated in a rotary flash evaporator (Roteva Equitron, Medica Instrument manufacturing company, Mumbai) under vacuum at a temperature not more than 50^0C and dried in a desiccator over anhydrous calcium chloride. The yield was found to be 4.72% w/v.

2.4. *Processing and storage*

Fresh plant materials were used for the pharmacognostic evaluation. The collected plant materials were dried in shade for about 15 days and powdered coarsely in the mill. The powder obtained was passed through # mesh # 40 and then used for physicochemical evaluation. The powders were extracted with Ethanol (95%) and the ethanolic extracts were used for phytochemical evaluation (Table 1).

2.5. *Phytochemical screening*

Phytochemical tests were carried out on the powdered sample using standard experimental procedures [6-9].

2.6. *Preparation of plates*

100 g of Silica gel-G was weighed and made into a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10 x 2, 10 x 5, 20 x 5, 20 x 10 cm etc.). The coated plates were allowed to dry in air, followed by heating at 100 – 105^{o} C for 1 h, cooled and stored in a dry atmosphere to protect from moisture. Before using, the plates were activated by heating at 100^{o} C for 10 min [10].

2.7. *Selection and maintenance of animals*

Wistar albino rats of either sex weighing 200-250 g were employed for the study. There were procured from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The rats were maintained under standard laboratory conditions at 25 ± 2^{0}C, relative humidity 50 ± 15% and normal photo period (12 h dark / 12 h light) were used for the experiment. Commercial pellet diet (Ratan Brothers, India) supplied by and water were provided when desired. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no.516/01/A/CPCSEA).

2.8. *Collection of blood samples*

The animal was restrained (unanaesthetised) in such a way that loose skin of the neck was tightened while handling the head with the left hand. With the help of the index finger the eye was pressed just behind the angle of the jaw resulting in the engorgement of the retro orbital plexus. Then tip of the capillary was inserted at the medical canthus into the retro-orbital plexus with gentle rotation by the other hand. As the vessels are ruptured, blood wells up in the peri-orbital space. The tip of the capillary was then slightly withdrawn, so that the blood flows into the capillary, which was collected in microcentrifuge tube containing small quantity of potassium oxalate and sodium flouride as an anticoagulant. Blood samples were collected retro-orbital plexus at 0, 2, 4, 6, 8, 12, 18 and 24 h. Blood glucose levels were estimated by GOD-POD method [11].

2.9. *Induction of diabetes and experimental design*

Animals were allowed to fast for 18 h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight intra peritoneally.

After confirmation of, diabetes rats (with blood sugar levels between250-350 mg/dl) were used for the experiment. Each group consisted of 5 animals. Group I normal rats treated with vehicle (1% Sodium CMC) and served as normal control, and Group VIII-X normal rats were treated with methanolic extract of *A. salvifolium* at a doses of 100, 250, and 500 mg/kg respectively, Group XI-XIII diabetic rats were treated with methanolic extract of *A. salvifolium* at doses of 100, 250 and 500 mg/kg respectively. Groups- XX and XXI were treated with Tolbutamide 40 mg/kg in normal and diabetic rats respectively. All the doses were administered orally [12].

2.10. *Statistical analysis*

All values were expressed as mean±SEM. The differences were compared using one-way analysis of variance (ANOVA) and Student's t test. *P* values <0.05 were considered as significant.

3. Results and Discussion

3.1. *Phytochemical characteristics*

The coarse powder of *A. salvifolium* (root) powder was brown in colour, which has characteristic odour and has bitter in taste. The extractive values of *A. salvifolium* root was 4.5% w/w with petroleum-ether, 3.2 % w/w with ahloroform, 6.4 % w/w with Ethanol and 4.6 % w/w with aqueous solvents. The loss on drying value was 12.8% w/w, the total ash value was 2.84% w/w, the acid-insoluble ash and water soluble ash were 1.24 and 0.8 % w/w, respectively. The extracts did not show any fluorescence. The ethanol extract gave total solid content of 91.96 % w/w. The percent yield with ethanolic extract gave 4.72 % w/w, which was semi solid, light green in colour with a bitter taste. It gave positive tests for phytosterols, triterpenes, flavonoids, carbohydrates and alkaloids. The extract was free from glycosides, saponins, tannins, proteins and amino Acids. The phytochemical characteristics of *A. salvifolium* root were shown in Table 1. TLC of *A. salvifolium* ethanolic extract with 5% H_2SO_4 in methanol as spraying agent and petroleum ether: chloroform (1:1) and chloroform: methanol (8:2) solvent system was shown in Fig. 1.

Table 1. Phytochemical characteristics of *A. salvifolium* (Root).

Physical tests of crude drugs	*Alangium salvifolium* (Root)
Nature	Coarse powder
Colour	Brown
Odour	Characteristic
Taste	Bitter

Table 1 (*contd*.)

Extractive values (% w/w)	4.5 (petroleum-ether)
	3.2 (chloroform)
	6.4 (ethanol)
	4.6 (aqueous)
Loss on drying (% w/w)	12.8
Total ash (% w/w)	2.84
Acid-insoluble ash (% w/w)	1.24
Water-soluble ash (% w/w)	0.8
Fluorescence analysis	No fluorescence
Total solid content (% w/w)*	91.96 (ethanolic extracts)
Yield % w/w	4.72
Nature	Semi solid
Colour	Light green
Odour	Characteristic
Taste	Bitter
Ethanolic extracts	
Phytosterols	+
Triterpenes	+
Glycosides	-
Saponins	-
Flavonoids	+
Tannins	-
Proteins and amino acids	-
Carbohydrates	+
Alkaloids	+

+ = Positive; - = Negative

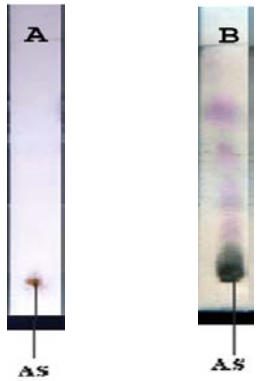

Fig. 1. TLC of *Alangium salvifolium* ethanolic extract.

3.2. *Effect of ethanolic extract of A. salvifolium roots on blood glucose levels in normal rats*

The mean blood glucose levels of control and extract treated animals after oral administration of different doses of ethanolic extract of *A. salvifolium* roots and Tolbutamide at various time intervals are shown in Table 2. The mean blood glucose levels at various time intervals were statistically evaluated in comparison with initial blood glucose level. The rats treated with 100mg/kg body weight of ethanolic extract of *A. salvifolium* roots produced highly significant reduction ($P < 0.001$) in blood glucose levels at 2, 4, 6 and 8 h while reduction was significant ($P < 0.05$) at 12, 18, and 24 h after oral administration of the extract. The glucose levels of animals treated with 250, and 500 mg/kg body weight were decreased significantly ($p < 0.001$) at 2, 4, 6, 8, 12 and 18h. Furthermore, the rats treated with standard drug Tolbutamide (40 mg/kg body weight) showed significant reduction in blood glucose up to 12 h.

The mean blood glucose levels produced by 100 mg/kg body weight of ethanolic extract of *A. salvifolium* were 88.44±1.95, 69.35±1.67, 52.36±1.21, 49.13±1.656, 52.95±1.06, 63.87±1.45, 70.52±1.21 and 79.16±0.69 mg/100ml at 0, 2, 4, 6, 8, 12, 18 and 24h, respectively, after the administration of 100 mg/kg body weight of ethanolic extract of *A. salvifolium* roots.

The mean blood glucose levels produced by 250 mg/kg body weight of ethanolic extract were 99.71±1.46, 76.0±0.74, 74.0±0.18, 80.6±0.56, 80.2±0.67, 88.3±0.53, 90.7±0.36 and 90.2±0.97 mg/100 ml at 0, 2, 4, 6, 8, 12, 18 and 24 h, respectively. Administration of 500 mg/kg body weight of ethanolic extract of *A. salvifolium* roots produced blood glucose levels of 97.32±0.94, 74.1±0.84, 69.2±0.64, 72.1±0.75, 76.4±0.48, 78. 2± 0.91, 87.8±0.53 and 89.4±1.75 mg/100ml at 0, 2, 4, 6, 8, 12, 18 and 24 h, respectively. The mean blood glucose levels after the administration of tolbutamide 40 mg/kg b.wt. were 96.55±0.71, 79.3±0.65, 65.2±0.61, 67.7±0.41, 71.3±0.46, 73.9±0.43, 87.8±0.52 and 88.1±0.24 mg/100ml at 0, 2, 4, 6, 8, 12, 18 and 24 h, respectively.

The lowest blood glucose levels were observed at 6^{th} h after oral administration of 100, 250 and 500 mg/kg body weight of ethanolic extract of *A. salvifolium* roots. The extract showed hypoglycaemic activity in dose dependant manner in normal fasting rats. The onset of hypoglycaemic action was found to be very quick and was sustained only up to 8^{th} h. The extract was found to be nearly as potent as the standard drug Tolbutamide in decreasing the blood glucose levels in normal fasting rats.

3.3. *Effect of ethanolic extract of A. salvifolium roots on percent decrease in blood glucose levels with respect to the control group*

The mean percent decrease in blood glucose levels produced by different doses of ethanolic extract at various time intervals compared with sodium CMC suspension treated control group are shown in Table 2. Oral administration of 100, 250 and 500 mg/kg body weight of ethanolic extract of *A. salvifolium* roots produced highly significant

($p < 0.001$) percent reduction in blood glucose at 2, 4, 6, 8 and 12 h compared to the control group at identical times. The standard drug tolbutamide showed highly significant ($P < 0.001$) decreasing blood glucose up to 12th h. The extent of percent reduction in blood glucose levels for the extract is nearly equal to that of standard.

Oral administration of 100 mg/kg body weight of ethanolic extract of *A. salvifolium* roots showed 21.58, 40.79, 44.44, 40.12, 27.78, 20.26 and 10.49 percent reduction in blood glucose at 2, 4, 6, 8, 12, 18 and 24 h compared with control group at identical times. The mean percent decrease in blood glucose levels produced by 250 and 500 mg/kg body weight of the ethanolic extract of *A. salvifolium* roots at 2, 4, 6, 8, 12, 18 and 24 h were 23.77, 25.77, 19.15, 19.55, 11.43, 9.02 and 23.84, 28.87, 25.89, 21.47, 19.32, 9.76 and 8.11, respectively.

Furthermore, the oral administration of the standard drug tolbutamide 40 mg/kg body weight showed 17.82, 32.43, 29.84, 26.11, 23.41, 9.01 and 8.70 percent reduction at 2, 4, 6, 8, 12, 18 and 24 h, respectively.

The maximum percent reduction in blood glucose was observed at 6th h as compared to the control group after oral administration of the ethanolic extract of *A. salvifolium* roots. The reduction in blood glucose levels was found to be dose dependant in normal fasting rats. The percent reduction in blood glucose was promising and statistically significant from 2nd h onwards sustained up to 12th h only indicating that the extract is fast acting. The extract showed significant decrease in blood glucose, when compared with the standard drug tolbutamide.

Table 2. Effect of oral administration of ethanolic extract of *A. salvifolium* on fasting blood glucose levels on normal rats.

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours							
		0	2	4	6	8	12	18	24
Control	-	93.51±1.12	91.4±1.33 (2.24)	90.4±1.31 (3.31)*	89.1±1.56 (4.70)	87.1±0.51 (6.09)	85.6±1.54 (8.44)	92.7±1.14 (0.85)	92.6±2.35 (0.96)
Alangium salvifolium	100	88.44±1.95	69.35±1.67 (21.58)	52.36±1.21 (40.79)	49.13±1.65 (44.44)	52.95±1.06 (40.12)	63.87±1.45 (27.78)	70.52±1.21 (20.26)	79.16±0.69 (10.49)
Alangium salvifolium	250	99.71±1.46	76.0±0.74 (23.77)	74.0±0.18 (25.77)*	80.6±0.56 (19.15)	80.2±0.67 (19.55)	88.3±0.53 (11.43)	90.7±0.36 (9.02)	90.2±0.97 (9.52)
Alangium salvifolium	500	97.32±0.94	74.1±0.84 (23.84)	69.2±0.64 (28.87)	72.1±0.75 (25.89)	76.4±0.48 (21.47)	78.2±0.91 (19.32)	87.8±0.53 (9.76)	89.4±1.75 (8.11)
Tolbutamide	40	96.55±0.71	79.3±0.65 (17.82)	65.2±0.61 (32.43)	67.7±0.41 (29.84)	71.3±0.46 (26.11)	73.9±0.43 (23.41)	87.8±0.52 (9.01)	88.1±0.24 (8.70)

All values are expressed as Mean± SEM; Values given in the brackets are percent blood glucose reduction.
*** $p < 0.001$ ** $P < 0.01$ * $P < 0.05$ statistically significant compared to 0h of their respective group.

3.4. *Effect of ethanolic extract of A. salvifolium roots on blood glucose levels in Alloxan induced diabetic rats*

The mean blood glucose levels were significantly evaluated after the intraperitoneal administration of Alloxan monohydrate. The mean blood glucose levels of control group and other group after oral administration of different doses of ethanolic extract of *A.*

salvifolium roots and Tolbutamide in Alloxan induced diabetic rats were shown in Table 3. The mean blood glucose levels were statistically evaluated by using student's 't' test in comparison to the mean blood glucose levels at 0 h. Oral administration of 0.5ml of 1% sodium CMC suspension to the diabetic rats didn't alter their blood glucose levels at all the time intervals. Oral administration of 100, 250 and 500 mg/kg body weight of ethanolic extract showed significant decrease ($P < 0.001$) in blood glucose levels up to 8th h. Tolbutamide showed significant decrease ($P < 0.001$, $P<0.05$) in glucose at the entire time interval. The mean blood glucose levels after the oral administration of 100 mg/kg body weight of ethanolic extract of *A. salvifolium* roots were 259.6±1.73, 214.1±16.11, 190.4± 3.48, 208.3±4.46, 224.1±6.78, 230.4±7.25, 247.3±6.73 and 248.9±4.74 mg/100ml. at 0, 2, 4, 6, 8, 12, 18 and 24 h, respectively. The mean blood glucose levels after administration of 250 and 500mg/kg body weight of ethanolic extract of *A. salvifolium* roots were 304.7±1.94, 226.6±2.81, 186.3±5.32, 218.6±5.59, 230.8±1.29, 42.3±8.75, 251.8±3.13 and 256.4±1.29 mg/100ml and 324.8±8.85, 246.7±1.81, 190.9±9.41, 218.0±4.28, 223.5±7.54, 248.7±6.77, 290.0±1.36 and 310.3±8.28 mg/100ml at 0, 2, 4, 6, 8, 12, 18h and 24 h, respectively. The mean blood glucose levels after the administration of Tolbutamide 40 mg/kg body weight were 368.5±14.81, 293.5±0.25, 225.7±1.25, 189.2±1.65, 222.1±9.85, 256.6±1.89, 290.4±2.14 and 302.3±5.21 mg/100ml. at 0, 2, 4, 6, 8, 12, 18 and 24 h, respectively.

The extract at all the dose levels i.e. at 100, 250 and 500 mg/kg body weight significantly lowered the evaluated blood glucose levels in Alloxan induced diabetic rats. The lowest blood glucose levels were observed at 4th h after oral administration of the different doses of ethanolic extract. Glucose levels were significantly decreased up to 24th h, where as the standard drug; Tolbutamide lowered the blood glucose level to the maximum at 6th h.

3.5. *Effect of ethanolic extract of A. salvifolium roots on percent decrease in blood glucose levels in Alloxan induced diabetic rats with respect to the control group*

The mean percent decrease blood glucose levels of control and the extract treated animals after oral administration of different doses of ethanolic extract of *A. salvifolium* roots at various time intervals are shown in Table 3.

The mean percent reduction in blood glucose levels was statistically evaluated in comparison to control group at identical time intervals. The mean percent decrease in blood glucose levels produced by all doses was significant ($P < 0.001$, $P < 0.05$) up to 12th h.

The mean percent decrease in blood glucose produced by 100 mg/kg body weight of ethanolic extract of *A. salvifolium* roots were 17.52, 26.65, 19.76, 13.67, 11.25, 4.73 and 4.12 at 2, 4, 6, 8, 12, 18 and 24 h, respectively. The mean percent decrease in blood glucose level after oral administration of 250 and 500 mg/kg body weight of ethanolic extract of *A. salvifolium* roots were 30.22, 38.85, 28.25, 22.6, 20.47, 17.36, 15.85 and 24.01, 41.22, 32.88, 31.23, 23.56, 10.71 and 4.46 at 2, 4, 6, 8, 12, 18 and 24 h,

respectively. The oral administration of the standard drug Tolbutamide 40mg/kg body weight showed 20.35, 38.74, 48.65, 39.72, 21.18 and 17.96 at 2, 4, 6, 8, 12, 18 and 24 h, respectively.

The extract at all dose levels showed promising, potent and statistically significant percent decrease in blood glucose. The percent reduction in blood glucose was significant and promising at 2nd h and gradually increased to the maximum level at 4th h and fallen back at 24th h. From these results it was found that the extract is having promising hypoglycaemic activity and more potent anti-hyperglycaemic activity in alloxan induced diabetic rats. Therefore it is quite obvious that the plant is found to be more potent and having rich traditional use.

Table 3. Effects of ethanolic extract of *A. salvifolium* on fasting blood glucose levels on diabetic rats.

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours							
		0	2	4	6	8	12	18	24
Control	-	277.4±15.12	270.7±9.62 (2.42)	268.5±9.56 (3.20)	265.2±5.93 (4.39)	265.5±7.88 (4.28)	268.6±5.48 (3.17)	273.4±8.94 (1.44)	279.7±9.36 (0.83)
Alangium salvifolium	100	259.6± 1.73	214.1±16.11 (17.52)	190.4±3.48 (26.65)	208.3±4.46 (19.76)	224.1±6.78 (13.67)	230.4±13.25 (11.25)	247.3±6.73 (4.73)	248.9±4.74 (4.12)
Alangium salvifolium	250	304.7±1.94	226.6±2.81 (30.22)	186.3±5.32 (38.85)	218.6±5.59 (28.25)	230.8±1.29 (22.6)	242.3±8.75 (20.47)	251.8±3.13 (17.36)	256.4±1.29 (15.85)
Alangium salvifolium	500	324.8±8.85	246.7±1.81 (24.01)	190.9±9.41 (41.22)	218.0±4.28 (32.88)	223.3±7.54 (31.23)	248.2±6.77 (23.56)	290.0±1.36 (10.71)	310.3±8.28 (4.46)
Tolbutamide	40	368.5±14.81	293.5±0.25 (20.35)	225.7±1.25 (38.74)	189.2±1.65 (48.65)	222.1±14.85 (39.72)	256.6±1.89 (30.35)	290.4±2.14 (21.18)	302.3±5.21 (17.96)

All values are expressed as Mean ± SEM; Values given in the brackets are percent blood glucose reduction.
*** p<0.001 ** P<0.01* P<0.05 Statistically significant compared to 0h of their respective group.

4. Conclusion

Alangium salvifolium root extract gave positive tests for phytosterols, triterpenes, flavonoids, carbohydrates and alkaloids. *A. salvifolium* root extract was found to be nearly as potent, faster onset and significantly decreases the blood glucose when compared with standard tolbutamide drug. The evaluated blood glucose levels in alloxan induced diabetic rats were significantly decreased up to 24th h compared to standard tolbutamide drug. The study revealed that ethanolic extract of *A. salvifolium* roots were found to have hypoglycaemic and anti-hyperglycaemic actions in normal and diabetic rats, respectively.

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