



Antioxidant and anti diabetic activities of leaf and flower extracts from *Acacia auriculiformis* A. Cunn. ex Benth.

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ABSTRACT

Introduction and Objective: *Acacia auriculiformis* A. Cunn. ex Benth. belongs to the Fabaceae family of flowering plants and has been reported to have useful medicinal properties such as those that control stomach diseases, aid contraception and facilitate abortion. It has recently been used as an alternative and complementary treatment for diabetes. Although there is considerable anecdotal information about the antidiabetic activity of this plant, much of this has not been substantiated by scientific evidence. The present study therefore, was aimed to investigate the antioxidant and antidiabetic activities of leaf and flower extracts from *Acacia auriculiformis* A. Cunn. ex Benth.

Methods: Leaves and flowers of *Acacia auriculiformis* A. Cunn. ex Benth. were extracted by macerating in 80% ethanol. Antioxidant activity of leaf and lower extracts (AALE and AAFE) from *Acacia auriculiformis* A. Cunn. ex Benth. was performed using DPPH scavenging analysis. The antidiabetic activity was carried out by a once daily oral application of the extracts at a dose of 250 mg/kg to normal and streptozotocin (65 mg/kg)-induced diabetic rats for 8 weeks to determine fasting blood glucose level, body weight, serum insulin, and blood chemistry in the rats. **Results:** The results revealed that AALE and AAFE possessed relatively low antioxidant activity compared to a standard ascorbic acid with IC₅₀ at 296.10±16.40 and 839.56±21.53 and 1.48±0.07 µg/ml respectively. The extracts significantly ($p < 0.05$) reduced fasting blood glucose, blood urea nitrogen (BUN), creatinine, albumin, alkaline phosphatase (ALP), and total protein, while significantly increasing serum insulin in the diabetic treated rats compared to the diabetic controls.

Conclusion: These findings indicate that the leaves and flower extracts from *Acacia auriculiformis* A. Cunn. ex Benth. possess antioxidant and antidiabetic activities that lower the fasting blood glucose level by increasing insulin secretion.

KEYWORDS : *Acacia auriculiformis* A. Cunn. ex Benth., diabetic rats, antidiabetic activity, antioxidant, serum insulin

1. Introduction

Acacia auriculiformis A. Cunn. ex Benth belongs to the Fabaceae family, and is one of the fastest-growing leguminous trees grown throughout tropical and subtropical regions. It has been reported to possess medicinal properties that control stomach diseases, facilitate abortion and provide contraception. It is one of the plants that have recently been used as an alternative complementary treatment for diabetes (1). The active fractions from *Acacia auriculiformis* A. Cunn. ex Benth leaf and flower have antidiabetic activity

(2). An aqueous extract derived from its boiled leaf and flower are taken orally to treat Type-2 diabetes. The leaves and flower extract from *Acacia auriculiformis* A. Cunn. ex Benth could inhibit elevated blood glucose and lipids level, and could increase the number of pancreatic islets per unit area.

(3). Many phytochemicals have been found in this plant. The leaves of *Acacia auriculiformis* A. Cunn. ex Benth contain phenolic compounds, aromatic amide and carboxylic acid

(4). The leaf and flower extract contains 44 compounds, but the principal constituents are 2 (H)-benzofuranone-5,6,7,7a-tetrahydro-4,4, 7a-trimethyl (1). Flavonoid quercetin was also isolated from the leaf and flower extract of *Acacia auriculiformis* A. Cunn. ex Benth

(5). The foliage and pods of *Acacia auriculiformis* A. Cunn. ex Benth contain mimosine, an amino acid known to be toxic to ruminants.

2. Objectives

The present study was carried out to investigate the antioxidant activity and antidiabetic activities of the leaf and flower extracts from *Acacia auriculiformis* A. Cunn. ex Benth

3. Methods

3.1 Plant materials: Leaves and flowers of *Acacia auriculiformis* A. Cunn. ex Benth were collected from Maha Sarakham Province, in northeastern Thailand. Voucher specimens were deposited in the Faculty of Pharmacy, Mahasarakham University, Thailand for reference.

3.2 Preparation of AALE and AAFE: The plant leaves and flowers were dried, powdered, extracted by maceration in 80% ethanol for 7 days, and then filtered. The filtrate was evaporated in a rotary evaporator to dryness.

3.3 Animals: Male albino Wistar rats weighing 250-300 g were used and housed under standard conditions (at 25±2°C, 50±5 % RH with a 12 h D/L cycle) and maintained with free access to water and a standard diet. The experimental protocol and performance of the rats were approved by the Institutional Ethical Committee for the Purpose of Control and Supervision on Experiment in Animals, Mahasarakham University, Thailand (License No. 0017/2011).

3.4 Induction of diabetes: Diabetes was induced by a single intra peritoneal injection of streptozotocin (65 mg/kg) (6). The rats with fasting blood glucose at or above 126 mg/dl were used as diabetic rats.

3.5 Antioxidant activity study: The antioxidant activity of the extracts was determined using colorimetric DPPH scavenging analysis (7).

3.6 Antidiabetic activity study: The rats were divided into 6 groups: Group 1 normal controls, Group 2 normal rats AALE treated, Group 3 normal rats AAFE treated, Group 4 diabetic controls, Group 5 diabetic rats 250 mg/kg AALE, and Group 6 diabetic rats 250 mg/kg AAFE. The extracts were given to the rats orally once daily for 8 weeks. The body weight and fasting blood glucose were measured

weekly. Eight weeks after administration the blood samples were determined for serum insulin and blood chemistry including blood urea nitrogen (BUN), creatinine, albumin, and alkaline phosphatase (ALP).

3.7 Statistical analysis: The data were analyzed using one way analysis of variance (ANOVA). The difference was taken to be significant at $p<0.05$. When the significant difference was noted, the Duncan's New Multiple Range Tests were performed. Results were expressed as the mean + SEM (n=8).

4. Results and Discussion

4.1 Antioxidant activity study

Analysis of AALE and AAFE with L-ascorbic acid, a positive control, revealed that the scavenging activity (IC50) of AALE and AAFE was relatively low compared to L-ascorbic acid (296.10+16.40, 839.56+21.53 and 1.48+0.07 µg/ml respectively)

4.2 Antidiabetic activity

4.2.1 Body weight - At the end of the experiments, the body weight of the normal control rats was significantly ($p<0.05$) higher than that of the diabetic controls. AALE did not affect the rats, but AAFE significantly ($p<0.05$) decreased the body weight of the normal rats; whereas, AALE and AAFE slightly increased the body weight of the diabetic treated rats

Table 1 Effects of AALE and AAFE on the body weight

Groups	Body weight (g)	
	initial	Final
Normal controls	296.66+5.57	365.50+12.50b
Normal rats + AALE	298.33+4.77	371.53+15.21b
Normal rats + AAFE	286.59+9.82	271.87+7.55a
Diabetic controls	291.00+7.63	262.50+8.81a
Diabetic rats + AALE	292.33+5.77	289.73+13.64a
Diabetic rats + AAFE	289.31+9.15	272.50+14.36a

Values are mean + SEM, n= 8 (per group). Within same column, values follow by different superscript indicate of significantly different between treatment ($p<0.05$)

4.2.2 Fasting blood glucose level - Initially, the fasting blood glucose level of the diabetic controls, the diabetic rats AALE treated, and the diabetic rats AAFE treated, was not different but significantly ($p<0.05$) higher than that of the normal controls, normal AALE treated rats and the normal AAFE treated rats. However, by the end of experiments, AALE and AAFE significantly ($p<0.05$) decreased the fasting blood glucose level in the diabetic rats. AALE and AAFE did not affect the body weight of the normal controls (Table 2). These data indicate AALE and AAFE possess anti diabetic properties confirming the anti diabetic activities of the extract and active fractions from *Acacia auriculiformis* A. Cunn. ex Benth in previous studies

(3). One of the mechanisms lowering the blood glucose level may involve flavonoid quercetin possessed in leaf extract .

Table 2 The effect of AALE and AAFE on fasting blood glucose

Groups	Fasting blood glucose (mg/dl)	
	Initial	Final
Normal controls	85.72+1.79a	88.11+6.13 a
Normal rats + AALE	83.57+8.45 a	81.23+5.87 a
Normal rats + AAFE	83.90+8.25 a	84.23+9.82 a
Diabetic controls	339.27+12.47d	406.85+12.39 e
Diabetic rats + AALE	336.25+11.19 d	218.98+14.61 c
Diabetic rats + AAFE	341.55+13.83 d	182.77+10.94 b

Values are mean + SEM, n= 8 (per group). Within same column,

values followed by different superscript indicate significant difference between treatments ($p<0.05$).

4.2.3 Serum insulin - Serum insulin was significantly ($p<0.05$) decreased in the diabetic control rats compared to normal controls. It increased but did not reach a statistical significance in the diabetic AALE and AAFE treated rats compared to that in the diabetic controls (Table 3). Decreasing blood glucose in the diabetic rats is likely due to an increase insulin secretion.

Table 3 Effects of AALE and AAFE on serum insulin

Groups	Serum insulin (U/ml)
Normal controls	21.04+5.23b
Normal rats + AALE	18.11+3.29ab
Normal rats + AAFE	18.56+6.35ab
Diabetic controls	13.75+2.96 a
Diabetic rats + AALE	16.51+5.43ab
Diabetic rats + AAFE	16.26+4.71ab

Values are mean + SEM (n= 8). Within same column, values follow by different superscript indicate of significantly different between treatment ($p<0.05$).

4.2.4 Blood chemistry -BUN, creatinine, albumin, ALP, and total protein were increased in the diabetic rats compared to controls. However, they were significantly ($p<0.05$) decreased in the diabetic AALE and AAFE treated rats compared to those in the diabetic controls (Table 4). These data suggest AALE and AAFE reduce renal and hepatic diabetic dysfunction. High doses or long term administration should be considered as the foliage and pods of *Acacia auriculiformis* A. Cunn. ex Benth contained mimosine, an amino acid known to be toxic to ruminants .

Table 4 Effects of AALE and AAFE on blood chemistry

Groups	Blood chemistry				
	BUN (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)	ALP (U/l)	TP (mg/dl)
Normal controls	30.02+1.04a	0.67+0.09a	3.91+0.15ab	90.12+10.44a	6.63+0.24ab
Normal rats + AALE	27.81+0.69a	0.79+0.11ab	3.76+0.57a	197.31+11.09b	6.61+0.37b
Normal rats + AAFE	26.75+0.76a	0.83+0.03b	3.60+0.77a	192.12+24.34b	6.86+0.20c
Diabetic controls	41.30+1.86b	0.90+0.04b	4.27+0.23c	242.00+12.15c	6.81+0.15bc
Diabetic rats + AALE	34.56+0.84a	0.86+0.53b	4.05+0.70b	236.17+15.58c	6.23+0.41a
Diabetic rats + AAFE	32.27+2.81a	0.88+0.03b	4.15+0.77bc	230.75+31.01bc	6.05+0.20a

Values are mean + SEM (n= 8). Within same column, values follow by different superscript indicate significant difference between treatment ($p<0.05$). BUN= blood urea nitrogen, ALP=alkaline phosphatase, TP=total protein

5. Conclusion

The leaf and seed extracts from *Acacia auriculiformis* A. Cunn. ex Benth have antioxidant and antidiabetic activities.

The antioxidant activity of these extracts is likely involved with an increase of insulin secretion. Research data suggests that AAFE reduced the blood glucose level but did not increase the body weight gain in diabetic rats. Creatinine, albumin, total protein, BUN, serum insulin, and pancreatic tissues in diabetic rats treated with AAFE were not different from normal rats. However, Hct, Hb, RBC and WBC, ALP, Cholesterol, Low density lipoprotein (LDL), High density lipoprotein (HDL) and Triglyceride were significantly (Significant Difference at $p<0.05$) higher than in normal rats and diabetic rats. The antioxidative capacity employing the 1,1-diphenyl-2-

picrylhydrazyl free radical (DPPH) assay revealed that AAFE possessed antioxidant activity with $EC_{50} = 2174.38 \pm 14.24 \mu\text{g/ml}$ compared to vitamin C $8.96 \pm 0.33 \mu\text{g/ml}$. Total phenolic content of AAFE was found to be $76.71 \text{ mg gallic acid/g}$. Catechin and Quercetin in ABSE was $217.70 \pm 1.77 \mu\text{g/g}$ and $348.89 \pm 3.07 \mu\text{g/g}$.

The future experiment should recommend on Toxicological test of *Acacia auriculiformis* A. Cunn. ex Benth flower extracts (AAFE) in male and female rats in order to evaluate the acute, sub acute and chronic toxicity in both sexes of experimental rats.

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7. References

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