Hypoglycemic and Hypolipidemic Properties of Leaf Extracts from *Phyllanthus acidus* (L.) Skeels., *Leucaena leucocephala* (Lam.) de Wit. and *Psidium guajava* (L.) in Streptozotocin-Induced Diabetic Rats

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***Abstract*— Star gooseberry *Phyllanthus acidus* (L.) Skeels, lead tree *Leucaena leucocephala* (Lam.) de Wit. and guava *Psidium guajava* (L.) are commonly well known plants and have been widely used in folk medicine for the treatment of many diseases in Thailand. Some pharmacological activities of leaf extracts from the plants in animal model of diabetes have been studied. To increase the pharmacological document of the plants, the present study was therefore carried out to investigate hypoglycemic and hypolipidemic properties of leaf extracts from *P. acidus, L. leucocephala* and *P. guajava*. The extracts at a dose of 250 mg/kg were administered to streptozotocin (65 mg/kg)-induced diabetic rats orally and daily for eight weeks. Blood glucose level, body weight, hematological values, lipid profiles, blood chemistry, and serum insulin in the rats were examined. Antioxidant activity of the extracts was also assessed by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. Moreover, to see whether the extracts have acute toxicity, once oral administration of the extracts at a dose of 1000, 1500 and 2000 mg/kg was performed in healthy rats. The results revealed that the extracts significantly ($p<0.05$) decreased blood glucose level, total cholesterol (TC), triglyceride, (TG), low density lipoprotein (LDL), blood urea nitrogen (BUN), and creatinine but increased high density lipoprotein (HDL) and serum insulin in the diabetic treated rats. However, hematological values including white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct) in both normal and diabetic rats were not affected by the extracts. DPPH assay revealed that the leaf extracts from *P. acidus, L. leucocephala* and *P. guajava* possessed the antioxidant activity with EC_{50} values of 232.37±15.27, 296.10±16.40 and 39.40±3.82 µg/ml respectively which was less potent than ascorbic acid (1.48±0.86 µg/ml). Moreover, the extracts at a dose up to 2000 mg/kg did not exhibit sign of acute toxicity as well as mortality of the rats within a period of observation.**

These findings indicate that the leaf extracts from *P. acidus, L. leucocephala* and *P. guajava* possess hypoglycemic and hypolipidemic activities. The activities seem to relate to hyperinsulinemia and antioxidant activities. Utilization of the extracts is safe with LD_{50}>2000 mg/kg. Furthermore, the leaf extract from *P. guajava* is to be the most effective extract in the study.

***Index Terms*— Phyllanthus acidus , Leucaena leucocephala, Psidium guajava blood glucose, blood chemistry**

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I. Introduction

Diabetes mellitus is a metabolic disease characterized by elevated blood glucose and resulted from either insufficient insulin and/or insulin resistance. The incidence of diabetes mellitus is tremendous increasing especially in developing countries. The disease causes substantial morbidity, mortality and long-term complications such as retinopathy, neuropathy and nephropathy. Maintaining near blood glucose concentration is mainly based on the use of hypoglycemic agent or insulin which has limited efficacy and associated undesirable side effects. Medicinal plants and natural products are considered to have less side effects, to be less toxic, easy to find, and low cost compared to synthetic drugs. The utilization of medicinal plants for alternative treatment of diabetes mellitus is now increasing. The potential role of some medicinal plants as hypoglycemic and hypolipidemic agents has been reported.

Phyllanthus acidus (L.) Skeels. is an edible small yellow berry fruit in the Phyllanthus family originated in Madagascar and commonly grown in Indonesia, South Vietnam, Loa, and Thailand in home gardens. Exhaustive literatures showed that *P. acidus* is a good remedy for various types of ailments including emetic and purgative [1], hypertension and respiratory [2], hepatoprotective [3], antidiabetic [4]. Its young leaves are cooked as a vegetable in Indonesia, India and Thailand [5]. The leaf is analgesic, antipyretics, antirheumatic and cures jaundice, small pox, itching and gum infection. An aqueous extract of leaf is reported to have remarkable antiviral [6], anticystic fibrosis [2], hepatoprotective and antioxidant properties [7].

Leucaena leucocephala (Lam.) de Wit belonged to the Fabaceae family is one of the fastest-growing leguminous and grown throughout tropical and subtropical regions. It has been reported to possess medicinal properties that control stomach diseases, facilitated abortion and provide contraction. It is one of the plants that alternative complementary treatment

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for diabetes [8]. Its leaf and seed extracts have been reported to possess antioxidant and antidiabetic activities [9]. The leaves have been reported to contain phenolic compounds, aromatic amide and carboxylic acid [10]. Its leaf extract contains 44 compounds, but the principal constituents are 2-(H)-benzofuranone-5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl [8]. Flavonoid quercitin was also isolated from the leaf extract [10]. The foliage of this plant has been found to contain mimosine, an amino acid known to be toxic to ruminants [11].

Psidium guajava (L.), the most widely cultivated *Psidium* species in family Myrtaceae is distributed worldwide in the tropical and subtropical areas. It is grown commercially for the fruits, known one of the riches sources of vitamin C. Various parts of the plant has been used in traditional medicine for the management of various conditions including malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums and a number of other conditions [12]. Flavonoids, gallic acid and tannin are invariably present in all parts of the plant [12]; [13]. The leaves are used in a traditional therapy for dysentery or diabetes [14]. The leaf extract was found to possess anticestodal [15], analgesic, anti-inflammatory properties [16], antimicrobial [17], hepatoprotective [14] and antioxidant activity [18]. The extract contains flavonoids, mainly quercetin derivatives [19]. Ethanolic extract from the leaves of *P. guajava* showed antioxidant capacity with the TEAC value of 4.908+0.050 mM/mg and the phenolic content in guava leaf fraction played a role on the antioxidant activity [20].

Although *Phyllanthus acidus, Leucaena leucocephala* and *Psidium guajava* have been widely used in the treatment of diabetes mellitus in folklore medicine in Thailand. Some pharmacological activities and the safety on the utilization of the extracts from these plants in the diabetic rats are still unclear. The purpose of this study was therefore designed to investigate hypoglycemic and hypolipidemic properties including fasting blood glucose levels, body weight, hematological values, lipid profiles, blood chemistry, and serum insulin in streptozotocin-induced diabetic rats treated with ethanolic leaf extracts from these plants. Moreover, to see whether the utilization of the plants is safe, acute toxicity of the extracts was investigated.

II. MATERIALS AND METHODS

A. Preparation of plant extracts

Fresh mature leaves of *P. acidus, L. leucocephala* and *P. guajava* were collected from home gardens in Maha Sarakham Province, Northeastern Thailand. The plant leaves were washed, cut into small pieces and dried in a hot air oven at a temperature of 50°C and then powdered. The powder was extracted by macerating in 80% ethanol (1:10 w/v) for 7 days. The mixture was filtered through a Whatman filter paper. Ethanol in the filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany). The obtained extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) were kept at a temperature of 4°C until being used.

B. Animal

Male *albino* Wistar rats weighing 200-250 g were the animal used in the study. The rats were kept in an air conditioned room at a temperature of 25±2°C, 12-h light/12-h dark cycle and relative air humidity of 40-60%. A standard chow and water were given to the rats *ad libitum*. They were acclimatized for 7 days prior to the commencing experiments. The rats were maintained in accordance with the guidelines of the Committee Care and Use of Laboratory Animal Resource, National Research Council Thailand and the advice of the Institutional Animal Care and Use Committee, Mahasarakham University, Thailand.

Induction of diabetes

The rats were induced to be diabetes by a single intra-peritoneal injection of 65 mg/kg streptozotocin, STZ (Sigma Chemicals, St. Louis, MO) dissolved in 20 mM citrate buffer pH 4.5. After STZ injection, the rats were provided with 2% sucrose solution as their drink for 48 h to alleviate the severity after initial hypoglycemic phase. Rats with blood glucose level at or above 200 mg/dl were considered to be diabetes and used in the study [21].

C. Pharmacological activities study

The rats were divided into 8 groups with 8 rats in each:
Group I; normal control rats treated with 0.5% tween 80
Group II-IV; normal rats treated with 250 mg/kg PAE, LLE and PGE respectively
Group V; diabetic control rats treated with 0.5% tween 80
Group VI-VIII; diabetic rats treated with 250 mg/kg PAE, LLE and PGE respectively

The extracts and 0.5%Tween 80 were administered to the rats orally and daily for eight weeks. Investigation blood glucose level, using Accu-chek Advantage II (Roche Germany), and body weight was performed weekly.

At the end of experiments, the rats were sacrificed by cervical dislocation technique. After an operation, the blood sample was then drawn from the rat hearts to examine hematological values, lipid profiles and blood chemistry using an automatic blood analyzer (Swelab Alfa, Biozen, Sweden). Serum insulin was also determined using a radioimmunoassay kit (MP Biomedicals-Orangeburg, USA) and detected by an automatic gamma counter (Wallac 1470 Wizard, Perkin Elmer instrument, Germany).

Antioxidant activity study

Determination of antioxidant activity of the extracts was carried out using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH solution was freshly prepared by dissolving 24 mg DPPH in 100 ml water, and stored at -20^{o}C until being used. Sample was allowed to react with DPPH for 24h in the dark condition. Absorbance was measured at 515 nm using spectrophotometer. The determinations were conducted in triplicate. The percentage inhibition of DPPH radical by the sample was calculated using the following formula

$$\%\text{inhibition} = [\{Abs_{control}-Abs_{sample}\}/Abs_{control}] \times 100$$

Acute toxicity study

The healthy rats were randomly divided into 10 groups with 8 rats in each:

Group I; rats treated with 0.5% Tween 80 (normal controls), Group II - IV; rats treated with 1000, 1500 and 2000 mg/kg PAE, Group V-VII; rats treated with 1000, 1500 and 2000 mg/kg LLE and Group VIII-X; rats treated with 1000, 1500 and 2000 mg/kg PGE respectively.

The extracts and 0.5%Tween 80 were once administered to the rats orally. Mortality and sign of toxicity including change in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity, change in gait, posture and response to handling were investigated individually 0.5, 2, 4, 8, 12, and 24 h after dosing, and a continue further period for 14 days. Furthermore, at the end of observation, the body weight of the rats was also investigated.

Statistical analysis

All the data were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using One-way ANOVA. The criterion for statistical significance was *p*-values less than 0.05.

III. Results

A. Pharmacological activities

Effect of PAE, LLE and PGE on blood glucose level

Table 1, an initial blood glucose level as well as final blood glucose level in the diabetic controls and diabetic treated rats was significantly ($p<0.05$) higher than those in normal controls and normal treated rats. However, the blood glucose level in the diabetic treated rats was significantly ($p<0.05$) lower than that in diabetic controls, while the blood glucose level in normal treated rats did not differ from that in normal controls.

Table 1 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on blood glucose level in normal controls, normal treated rats, diabetic controls and diabetic treated rats.

Groups	Blood glucose level (mg/dl) Initial	Final
Normal controls	86.17±4.36 [a]	88.61±3.72 [a]
Normal rats + PAE	83.56±3.80 [a]	79.81±2.86 [a]
Normal rats + LLE	82.68±7.12 [a]	78.86±3.52 [a]
Normal rats + PGE	83.45±3.16 [a]	81.62±3.78 [a]
Diabetic controls	339.58±14.81 [c]	406.85±12.39 [d]
Diabetic rats + PAE	334.72±12.69 [c]	312.19±13.06 [b]
Diabetic rats + LLE	346.01±15.37 [c]	322.98±14.61 [b]
Diabetic rats + PGE	351.52±12.11 [c]	295.76±13.17

B. Effect of PAE, LLE and PGE on body weight

Table 2, an initial body weight of all rat groups was not different. However, at the end of experiments, the body weight of the diabetic groups was significantly ($p<0.05$) lower than normal groups. The extracts had no effect on body weight when the body weight of the treated groups was not different from control groups.

Table 2 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on body weight in normal controls, normal treated rats, diabetic controls and diabetic treated rats.

Groups	Body weight (g) Initial	Final
Normal controls	264.79±12.81 [a]	387.06±11.72 [b]
Normal rats + PAE	262.51±11.34 [a]	376.49±12.36 [b]
Normal rats + LLE	260.98+13.07 [a]	381.44±10.98 [b]
Normal rats + PGE	261.89±34.62 [a]	368.52±27.81 [b]
Diabetic controls	262.13±15.43 [a]	236.25±12.91 [a]
Diabetic rats + PAE	261.83±11.59 [a]	247.63±13.28 [a]
Diabetic rats + LLE	263.98±10.86 [a]	273.87±11.91 [a]
Diabetic rats + PGE	260.89±19.28 [a]	271.56+17. 34

C. Effect of PAE, LLE and PGE on lipid profiles

Cholesterol, triglyceride and LDL significantly ($p<0.05$) increased while HDL significantly ($p<0.05$) decreased in the diabetic controls compared to those in normal controls. However, they were reversed in the diabetic treated groups. PAE recovered the elevated triglyceride and LDL in diabetic treated groups closely to those in normal controls. The extracts slightly increased HDL in the diabetic treated rats when compared to that in diabetic controls (Table 3).

Table 3 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on lipid profiles in normal controls, normal treated rats, diabetic controls and diabetic treated rats.

Groups	Lipid profiles (mg/dl) Cholesterol	Triglyceride	LDL	HDL
Normal controls	49.25±2.05 [a]	102.75±6.61 [a]	6.03±2.54 [b]	46.50±2.34 [b]
Normal rats + PAE	39.50±1.20 [a]	108.81±5.92 [a]	4.76±2.91 [a]	40.31±3.29 [ab]
Normal rats + LLE	46.29±3.98 [a]	111.28±6.67 [a]	4.92±7.84 [a]	42.83±4.81 [ab]
Normal rats +PGE	48.86±7.95 [a]	104.31±7.46 [a]	6.20±8.11 [b]	44.56±7.07 [b]
Diabetic controls	91.83±7.30 [c]	247.33±24.32 [c]	8.91±6.25 [c]	34.81±6.19 [a]
Diabetic rats + PAE	58.40±3.01 [b]	115.80±9.82 [a]	4.65±3.29 [a]	36.37±7.86 [a]
Diabetic rats + LLE	65.66±3.26 [b]	192.54±8.87 [b]	6.52±7.81 [b]	34.47±3.96 [a]
Diabetic rats + PAE	76.22±9.87 [b]	219.37±5.49 [b]	7.79±6.03 [b]	38.81 ±4.59 [a]

LDL = Low density lipoprotein, HDL= High density lipoprotein

D. Effect of PAE, LLE and PGE on serum insulin

Table 4, serum insulin significantly ($p<0.05$) decreased in the diabetic controls compared to that in normal controls. The extracts significantly ($p<0.05$) increased serum insulin in the diabetic treated groups closely to that in normal controls. However, the extracts could not produce any change in normal treated rats compared to normal controls.

Table 4 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on serum insulin in normal controls, normal treated, rats diabetic controls and diabetic treated rats.

Groups	Serum insulin (µIU/m)l
Normal controls	24.75±1.13[c]
Normal rats + PAE	23.79±1.81[c]
Normal rats + LLE	22.39+2.38[c]

Normal rats + PGE	23.83±2.92[c]
Diabetic controls	13.97±1.49[a]
Diabetic rats + PAE	19.52±2.38[b]
Diabetic rats + LLE	51.00+5.43[b]

E. Effect of PAE, LLE and PGE on hematological values

Hematological values including hematocrit (Hct), hemoglobin (Hb), red blood cell (Rbc), and white blood cell (Wbc) in diabetic controls and normal controls were not different. And also, these parameters found in diabetic treated were not different from those in normal treated groups (Table 5).

Groups	Hematological values			
	Hct (%)	Hb (g%)	Rbc ($x10^6$cell/ml)	Wbc ($x10^3$cell/ml)
Normal controls	44.65±0.14[a]	14.22±1.64[a]	8.20±1.02[a]	4.70±0.52[a]
Normal rats + PAE	46.95±1.63[a]	14.98±3.87[a]	8.41±1.07[a]	3.90±0.32[a]
Normal rats + LLE	48.86+7.23[a]	15.61+3.91[a]	8.93+1.36[a]	4.21+2.97[a]
Normal rats + PGE	41.39±1.38[a]	14.57±1.49[a]	7.22±1.57[a]	4.63±0.93[a]
Diabetic controls	45.67+3.66[a]	15.35±0.92[a]	8.15±1.22[a]	3.34±1.26[a]
Diabetic rats + PAE	47.96±1.82[a]	16.89±2.38[a]	8.67±1.81[a]	3.94±1.22[a]
Diabetic rats + LLE	44.18±1.39[a]	16.08±3.81[a]	9.12±3.87[a]	3.51±2.40[a]
Diabetic rats + PGE	44.50±7.01[a]	16.50±1.67[a]	7.75±1.17[a]	4.22±1.00

Hct = hematocrit, Hb = hemoglobin, Rbc = red blood cell, Wbc = white blood cell

F. Effect of PAE, LLE and PGE on blood chemistry

Blood urea nitrogen (BUN) and alkaline phosphatase (ALP) were significantly ($p<0.05$) increased in the diabetic controls compared to those in normal controls. In contrast, they were significantly ($p<0.05$) decreased in the diabetic treated groups compared to those in diabetic controls. PAE significantly ($p<0.05$) decreased BUN and ALP in normal treated groups compared to those in normal controls. PAE and PGE significantly ($p<0.05$) decreased creatinine in diabetic treated rats compared to that in diabetic controls. However, the extracts could not alter creatinine in both normal and diabetic treated rats (Table 6).

Table 6 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on blood chemistry (BUN, creatinine and ALP) in normal controls, normal treated rats, diabetic controls and diabetic treated rats.

Groups	Blood chemistry		
	BUN (mg/dl)	Creatinine (mg/dl)	ALP (IU/L)
Normal controls	31.10+2.97[b]	0.72+1.23[bc]	132.94+3.49[a]
Normal rats + PAE	24.53±1.82[a]	0.53±1.92[b]	186.14±2.11[b]
Normal rats + LLE	27.81+0.69[a]	0.79+0.11[bc]	197.31+1.09[b]
Normal rats + PGE	29.43+1.24[b]	0.73+1.04[bc]	129.78+5.82[a]
Diabetic controls	41.30+1.86[c]	0.57±1.26[b]	250.36±5.65[d]
Diabetic rats + PAE	29.38±2.33[b]	0.49±1.41[a]	136.87±5.93[a]
Diabetic rats + LLE	34.56+0.84[bc]	0.86 +0.53[c]	236.17+5.58[c]
Diabetic rats + PGE	26.22+1.93[a]	0.27+1.18[a]	125.00+4.78[c]

BUN = blood urea nitrogen, ALP = alkalinephosphatase

G. Antioxidant activity of PAE, LLE and PGE

PAE and LLE exhibited significantly ($p<0.05$) and relatively low antioxidant activity (EC_{50} = 232.37±15.27 and 296.10=16.40 µg/ml respectively) compared to PGA (EC_{50}= 39.40±3.82 µg/ml) in DPPH assay. Reference standard ascorbic acid exhibited antioxidant activity with an EC_{50} of 1.48+0.86 µg/ml (Table 7).

Table 7 Antioxidant activity of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) using DPPH assay.

Extracts	Antioxidant activity EC_{50} (µg/ml)
PAE	232.37±15.27[c]
LLE	296.10±16.40[d]
PGE	39.40±3.82[b]
Ascorbic acid	1.48+86[b]

H. Acute toxicity

After a single administration of the extracts to the rats, sign of toxicity and mortality were not found during a period of observation for 24 h and a further 14 days. An initial body weight as well as final body weight of all groups was not different. However, the rat body weight was significantly ($p<0.05$) increased depending on age (Table 8).

Table 8 Effect of the leaf extracts from *P. acidus* (PAE), *L. leucocephala* (LLE) and *P. guajava* (PGE) on body weight of normal controls and treated rats after once oral administration of the extracts to investigate acute toxicity.

Groups		Body weight (g)	
		Initial	Final
	Normal controls	180.00±3.13[a]	205.63±2.00[b]
PAE			
	1000 mg/kg	180.00±2.67[a]	203.25±2.25[b]
	1500 mg/kg	183.13±8.81[a]	210.63±7.03[b]
	2000 mg/kg	181.38±8.00[a]	210.00±7.01[b]
LLE			
	1000 mg/kg	182.26±1.27[a]	207.12±3.21[b]
	1500 mg/kg	180.83±3.51[a]	204.24±6.11[b]
	2000 mg/kg	181.19±5.09[a]	209.60±3.71[b]
PGE			
	1000 mg/kg	182.06±1.67[a]	210.06±3.19[b]
	1500 mg/kg	181.41±7.11[a]	209.13±3.54[b]
	2000 mg/kg	181.36±5.63[a]	208.93±4.86[b]

IV. Discussion

Phyllanthus acidus, *Leucaena leucocephala* and *Psidium guajava* are commonly found and used as vegetable and as well as traditional medicine in Thailand. Since pharmacological activities in animal model of diabetes and toxicological study of these plants are poorly documented. The present study was therefore carried out by investigation hypoglycemic and hypolipidemic properties and also acute toxicity of the extracts to ratify theirs traditional used for the treatment of diabetes.

Pharmacological property study revealed that repeated administration of the extracts at a dose of 250 mg/kg to the rats for eight weeks possessed hypoglycemic and hypolipidemic activities of the extracts by significantly decreasing blood glucose level, cholesterol, triglyceride and LDL while significantly increasing serum insulin and HDL in the diabetic treated rats. However, serum insulin in the diabetic treated rats is still significantly lower than that in

normal controls indicating the slightly potent hyperinsulinemia activity of the extracts. Hct, Hb, Rbc, and Wbc were not different among the rat groups. Indicating, the diabetic state and the extracts have no effect on hematological values. The extracts recover the pathology of renal function by lowering BUN, creatinine and ALP in the diabetic treated groups compared to diabetic controls. However, LLE significantly increased creatinine in both normal and diabetic rats compared to normal controls. Antioxidant activity study revealed that PGE possess potent antioxidant activity higher than PAE and LLE respectively. Nevertheless, the antioxidant activity of the extracts was relatively low when compared to the standard ascorbic acid. Acute toxicity study showed that adverse effect and mortality were not observed beyond 14 days observation period indicating that the leaf extracts from *P. acidus*, *L. leucocephala* and *P. guajava* at doses up to 2000 mg/kg are safe for oral toxicity investigation.

In conclusion, the leaf extracts from *Phyllanthus acidus*, *Leucaena leucocephala* and *Psidium guajava* possess hypoglycemic and hypolipidemic properties and can be used safely for the treatment of diabetes without affecting on hematological values and renal function. Hypoglycemic and hypolipidemic properties of the extracts seem to relate to hyperinsulinemia and antioxidant activities.

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