ISSN 2278-7763

BIOACTIVITIES of ETRACTS from *Tinospora crispa* STEMS, *Annona squamosa* LEAVES, *Musa sapientum* FLOWERS, and *Piper sarmentosum* LEAVES in DIABETIC RATS

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ABSTRACT

The present study was performed to investigate the activities of the Thai medicinal plant extracts from T. crispa stems, A. squamosa leaves, M. sapientum flowers, and P. sarmentosum leaves on haematological values and bioactivities in streptozotocin (65 mg/kg)-induced diabetic rats (6 weeks). An acute toxicity of the extracts was also examined by oral administration at 1000, 1500 and 2000 mg/kg. The results revealed that the extracts (250 mg/kg) decreased the blood glucose level but increased the body weight of rats. However, the extracts did not produce any alteration of chemical properties of urine in the diabetic rats. Moreover, decreasing of white blood cell, red blood cell, hemoglobin, hematocrit, total cholesterol, triglyceride, low density lipoprotein, blood urea nitrogen, and creatinine, but increasing in mean corpuscular volume, mean corpuscular hemoglobin, lymphocytes, monocytes, high density lipoprotein, and serum insulin, and no altering of mean corpuscular hemoglobin concentration were recorded in the diabetic treated rats. The extracts have non-acute toxicity within 24 h and 14 days after administration (LD_{50}>2000 mg/kg), but possess hypoglycemic, hypolipidemic and hyperinsulinemic activities. The stem extract from T. crispa is likely to be the most effective extract on lowering the blood glucose level in this study via increasing serum insurin.

Keywords : *Tinospora crispa, Annona squanosa, Musa sapientum, Piper sarmentosum, Blood glucose level, haematological values, Blood chemistry*

1 INTRODUCTION

DIABETES mellitus is a group metabolic disease characterized by elevated blood glucose resulting from either insufficient insulin or insulin resistance. The incidence of diabetes mellitus is tremendous increasing in the most part of the world, especially in developing countries. This 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 oral hypoglycemic agent and insulin which has limited efficacy and associated undesirable side effects. Plant drugs are frequently considered to have fewer side effects, to be less toxic, easy to find and low cost compared to synthetic drugs. The use of medicinal plants for alternative treatment of diabetes mellitus is now increasing. The potential role of medicinal plants as hypoglycemic agents has been reported. ***Tinospora crispa*** is a climber plant widely distributed in Indonesia, Malaysia, Thailand and Vietnam and has long been used as a medicine. The whole plant contains a bitter principle, columbine, traces of an alkaloid, and a glycoside. *T. crispa* is one ingredient in Thai folk remedies for maintaining good health. A decoction of the stems, leaves and roots is used to treat fever, cholera, diabetes, rheumatism and snake-bikes. ***Musa sapientum,*** commonly known as banana, is grown in Thailand and South India for its flowers, fruits, stems, roots, and leaves. It is mainly used in Indian folk medicine for a treatment of diabetes mellitus. The juice of *M. sapientum* flowers is claimed to have beneficial effects in reducing blood sugar by local practitioners. Various parts of *M. sapientum* have been used for various medicinal purposes including the treatment of diabetes mellitus. ***Annona squamosa*** is a multiplepurpose tree with edible fruits. It is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, ruinting worm infestation, constipation, hemorrhage, dysuria, fever, thirst, malignant tumors and ulcers and also as an abortifacient [1]. Alkaloids present in *A. squamosa* has proved to have antioxidant activity [1], [2]. The ethanolic extract of the leaves and stems is reported to have anticancer activity [3]. Flavonoid and plant extracts have been showed remarkable antimicrobial and cytotoxic activities [4], [5]. ***Piper sarmentosum*** is a glabrous, creeping terrestrial herb. The plant and fruits are used in Thailand as an expectorant [6]. An ethanolic leaf extract has been reported to reduce blood sugar in alloxan diabetic rabbits [7]. In Malay and Indonesian Archipalago, the leaves and roots of this plant are used for the treatment of toothache, fungoid dermatitis on the feet, coughing asthma and pleurisy [8]. The extract of the whole plant showed a hypoglycemic effect in rats [9].

Although these plants have been widely used in the treatment of diabetes mellitus in folklore medicine in Thailand. However, toxicity and the influence of the extracts of these plants in the diabetic rats were still unclear. The purposes of this study were therefore designed to investigate acute toxicity of these extracts, fasting blood glucose levels, body weight, hematological values, blood chemistry, and serum insulin in streptozotocin-induced diabetic rats treated with ethanolic extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, or *P. sarmentosum* leaves.

ISSN 2278-7763

2 MATERIALS and MTHODS

2.1 Preparation of Plant Extracts

The plant parts; *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves were purchased from a local market in Loei Province, Northeastern Thailand. The specimens were identified by the Plant Varieties Protection Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The voucher specimens are deposited in the Department of Biology, Faculty of Science, Mahasarakham University, Thailand. The fresh specimens were washed, cut into small pieces and dried in a hot air oven at 50°C. Dried specimens were powdered and 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 obtained filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany). The extracts were kept at -20°C until being used.

2.2 Animal

Male *albino* Wistar rats weighing 200-250 g purchased from the National Laboratory Animal Centre (NLAC), Mahidol University, Thailand were used in the study. The rats were acclimatized in an air conditioned room at 25±2°C, 12-h light/12-h dark cycle and relative air humidity of 40-60% , and given a standard chow and watered *ad libitum* 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.

2.3 Induction of siabetes

The rats were injected intraperitonealy using a single dose of 65 mg/kg streptozotocin, STZ (Sigma Chemicals, St. Louis, MO) dissolved in freshly and cool 20 mM citrate buffer pH 4.5 (Talubmook, Forrest and Parsons, 2003). After an injection, the rats were provided with 2% sucrose solution as their drink for 48 h to alleviate the severity after initial hypoglycemic phase. Three days after injection, the rats were examined to FBG for confirm diabetic stage. Rats with FBG at and higher than 126 mg/dl were considered to be diabetes [10].

2.4 Experimental Design

2.4.1 Acute toxicity study

The healthy rats were randomly divided into 13 groups with 8 rats in each: group I; rats treated with 0.5% tween 80 (normal control), group II to XIII; rats treated with various doses (1000, 1500 and 2000 mg/kg) of the extracts from *T. crispa* stem, *A. squamosa* leaves, *M. sapientum* flowers, *and P. sarmentosum* leaves respectively.

The extracts were suspended in 0.5% tween 80 and once administered to the rats orally. Sign of toxicity and a number of mortal rats were investigated within 24 hours and a further period for 14 days after the administration.

2.4.2 Blood Glucose Level, Hematological Values, Chemical Properties of Urine, Blood Chemistry, and Insulin Level

The rats were divided into 7 groups with 8 rats in each: group I; normal control rats treated with 0.5% tween 80, group II; diabetic control rats treated with 0.5% tween 80, group III; diabetic rats treated with 0.25% glibenclamide, group IV to VII; diabetic rats treated with 250 mg/kg of the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves respectively.

The extracts and glibenclamide were administered to the rats orally and daily for six weeks using an orogastric tube. Blood sample was collected by taken from the tail vein of the fasted overnight rats. The fasting blood glucose (FBG) was measured weekly using Accu-chek Adventage II (Roche Germany). Chemical properties of urine were measured week after week using Combur^{10} Test®UX test strip (Roche Diagnostics GmbH, D-68298 Mannheim, Germany).

At the end of experiments, the rats were fasted overnight and sacrificed by cervical dislocation technique. After an operation, the blood sample then was drawn from the rat heart to examine hematological values, blood chemistry, using an automatic blood analyzer (Swelab Alfa, Biozen, Sweden), and to assess serum insulin which was performed after blood sample was centrifuged to separate blood serum. Serum insulin was estimated using a radioimmunoassay kit (MP Biomedicals-Orangeburg, USA) and detected by an automatic gamma counter (Wallac 1470 Wizard, Perkin Elmer instrument, Germany).

2.4.3 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

3 RESULTS

3.1 Acute toxicity

After a single oral administration of the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves to the rats, sign of toxicity and mortal rats were not found within a period of observation.

3.2 Blood glucose levels

The blood glucose level in the diabetic controls was significantly ($p<0.05$) increased compared to normal controls (data not shown). However, at the end of experiments, the blood glucose level was lowered by 49.57±4.85, 26.21±3.23, 32.15±4.84, and 45.24±5.46% in the diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves respectively. The stem extract from *T. crispa* significantly ($P<0.05$) produced the percentage reduction of the blood glucose level greater than that from *A. squamosa* leaves, but this was not greater than those from *M. sapientum* flowers, and *P. sarmentosum* leaves in Figure 1.

3.3 Body weight

The body weight of the diabetic controls was significantly ($p<0.05$) decreased compared to normal controls (data not shown). The extracts from *T. crispa* stems, *M. sapientum* flowers and *P. sarmentosum* leaves significantly ($p<0.05$) increased the body weight of the diabetic treated rats compared to dia-

ISSN 2278-7763

betic controls. The extract from *P. sarmentosum* leaves produced the percentage increasing in the body weight of the diabetic treated rats greater than those from from *T. crispa* stems, *M. sapientum* flowers and *A. squamosa* leaves respectively in Figure 2.

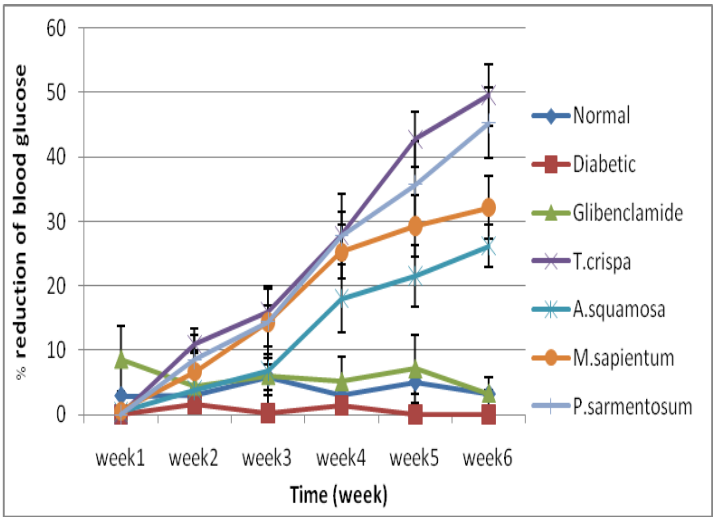

Figure 1 Percentage reduction of the blood glucose level in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

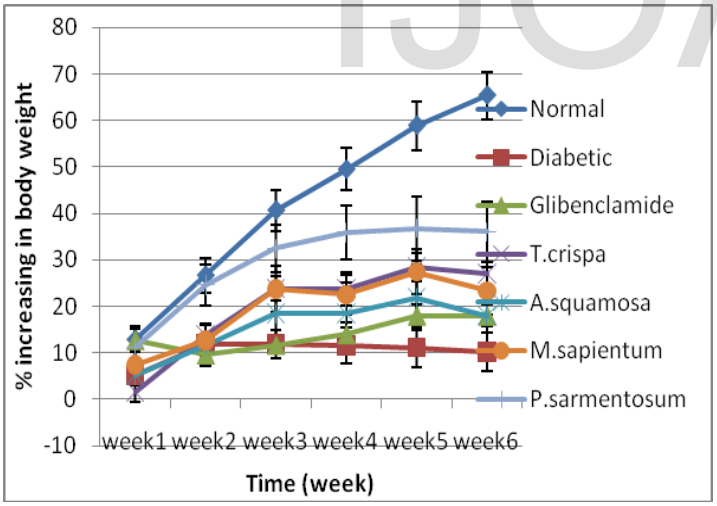

Figure 2 Percentage increasing in the body weight of normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

3.4 Hematological values

Hemoglobin (Hb), red blood cell count (Rbc) and white blood cell count (Wbc) were significantly ($p<0.05$) increased, while platelet was significantly ($p<0.05$) decreased in the diabetic controls compared to those in normal controls.

The extract from *T. crispa* stems *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves reduced haematocrit (HCt), hemoglobin (Hb), red blood cell (RBC), and white blood cell (WBC) but increased platelet in the diabetic treated rats compared to diabetic controls. The extract from *T. crispa* stems and *A. squamosa* leaves significantly ($p<0.05$) reduced haematocrit (HCt), hemoglobin (Hb), red blood cell (RBC), and white blood cell (WBC) but increased platelet in the diabetic treated rats closely to normal controls in Table 1.

TABLE 1

Table 1 Hematological values in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

Animal groups	Hematological values				
	HCt (%)	Hb (g/dl)	RBC ($x10^6$ cell/mm^3)	WBC ($x10^3$cell /mm^3)	PLT ($x10^3$/mm^3)
Normal controls	48.67± 0.41^{ab}	16.53± 0.17^{a}	7.92± 0.54^{b}	1.60± 0.08^{a}	553.50± 32.99^{b}
Diabetic controls	51.17± 0.79^{b}	17.85± 0.28^{b}	8.62± 0.16^{c}	2.12± 0.11^{b}	476.17± 20.01^{a}
Glibenclamide	50.41± 0.35^{ab}	17.52± 0.19^{ab}	6.96± 0.02^{a}	1.75± 0.07^{a}	487.65± 34.26^{a}
T. crispa	47.67± 0.56^{a}	16.43± 0.11^{a}	7.93± 0.02^{b}	1.38± 0.14^{a}	532.33± 58.97^{b}
A. squamosa	47.67± 0.42^{a}	16.35± 0.05^{a}	8.11± 0.12^{bc}	1.98± 0.09^{ab}	554.17± 5.74^{b}
M. sapientum	48.00± 0.57^{a}	16.75± 0.12^{ab}	7.85± 0.03^{b}	1.87± 0.02^{ab}	497.00± 16.52^{a}
P. sarmentosum	51.17± 0.77^{b}	16.50± 0.29^{a}	8.41± 0.07^{c}	1.60± 0.04^{a}	507.00± 8.84^{ab}

Data were expressed as mean ± S.E.M. of six rats. Within a column, the mean values flowed by a different letters were significantly different ($P<0.05$)analyzed using DMRT)HCt =Hematocrit, Hb=Hemoglobin, RBC =Red blood cell, WBC =White blood cell, PLT= platelet).

3.4.1 Differential white blood cell count

Lymphocytes and Monocytes were reduced in the diabetic controls compared to normal controls. However, they were increased in the diabetic rats treated with the extracts. The extract from *M. sapientum* flowers and *T. crispa* stems increased number of lymphocytes and monocytes closely to those in normal controls in Table 2.

ISSN 2278-7763

TABLE 2

Differential white blood cell count in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

Animal groups	Differential white blood cell count	
	Lymphocyte ($x10^3/mm^3$)	Monocyte ($x10^3/mm^3$)
Normal controls	94.15±0.97^{b}	10.26±0.97^{a}
DM controls	59.97±4.85^{a}	9.25±4.85^{a}
Glibenclamide	87.65±4.97^{b}	8.81±6.87^{a}
T. crispa	93.12±1.19^{b}	12.27±1.19^{ab}
A. squamosa	79.58±4.15^{ab}	9.60±4.51^{a}
M. sapientum	95.07±0.75^{b}	10.25±0.75^{a}
P. sarmentosum	73.90±6.67^{ab}	14.28±6.67^{b}

Data were expressed as mean ± S.E.M. of six rats. Within a column, the mean values flowed by a different letters were significantly different (P<0.05)analyzed using DMRT.

3.4.2 Serum insulin

Serum insulin was significantly (p<0.05) reduced in the diabetic controls compared to normal controls. However, it was increased in the diabetic treated rats. The extracts from *T. crispa* stems and *P. sarmentosum* leaves significantly (p<0.05) increased serum insulin in the diabetic treated rats compared to diabetic controls in Figure 3.

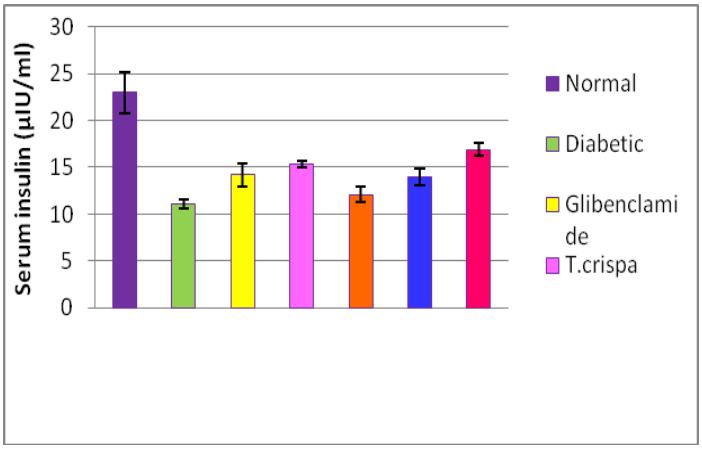

Figure 3 Serum insulin in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves.

TABLE 3

Lipid profiles in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

Animal groups	Lipid profiles (g/dl)			
	Cholesterol	Triglyceride	HDL	LDL
N- controls	84.33±2.43^{a}	85.33±4.94^{a}	51.00±1.02^{c}	16.33±2.11^{a}
DM controls	165.33±2.71^{c}	198.83±5.74^{c}	23.83±0.77^{a}	101.73±2.88^{d}
Glibenclamide	93.70±2.37^{a}	149.70±4.87^{b}	28.30±0.69^{a}	35.30±2.19^{b}
T. crispa	118.50±4.82^{ab}	104.33±9.60^{a}	47.67±1.56^{c}	49.97±3.28^{bc}
A. squamosa	161.50±5.40^{c}	182.33±9.99^{c}	40.50±3.10^{b}	84.53±2.63^{c}
M. sapientum	139.50±2.00^{b}	187.17±5.79^{c}	37.00±1.48^{b}	65.07±3.58^{c}
P. sarmentosum	138.50±3.65^{b}	110.00±7.98^{a}	40.67±1.33^{b}	75.83±3.35^{c}

Data were expressed as mean ± S.E.M. of six rats. Within a column, the mean values flowed by a different letters were significantly different (P<0.05)analyzed using DMRT.

TABLE 4

Blood urea nitrogen, creatinine and serum insulin in normal controls, diabetic controls, diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves

Animal groups	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)
N- controls	29.00±0.97^{a}	0.62±0.02^{bc}
DM -controls	39.83±2.14^{c}	0.65±0.02^{c}
Glibenclamide	32.63±0.84^{b}	0.56±0.02^{b}
T. crispa	32.83±0.95^{b}	0.50±0.04^{b}
A. squamosa	37.67±1.43^{c}	0.52±0.02^{b}
M. sapientum	35.67±1.23^{bc}	0.47±0.02^{a}
P. sarmentosum	34.50±1.48^{bc}	0.60±0.03^{bc}

Data were expressed as mean ± S.E.M. of six rats. Within a column, the mean values flowed by a different letters were significantly different (P<0.05)analyzed using DMRT.

ISSN 2278-7763

In line with lipid profiles, blood urea nitrogen (BUN) and creatinine were increased in the diabetic controls but reversed in the diabetic treated rats in Table 4.

3.4.3 Chemical properties of urine

The chemical properties of urine including specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin in normal controls, diabetic controls and diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves were not different. However, glucose found in the urine of diabetic controls was still found in those in the diabetic treated rats (data not shown).

4 DISCUSSION AND CONCLUSIONS

The extracts at the highest dose (2000 mg/kg) using in the present study did not produce sign of toxicity and mortality in the rats. Therefore, the LD_{50} of these extracts is higher than 2000 mg/kg. This result is in line with a study by [11] who study on acute and sub-acute toxicities of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf extract and found that all doses of the extracts used (500, 1000, 1500, and 2000 mg/kg) did not produce sign or symptom of acute and sub-acute toxicities, and also the mortality of rats was not observed.

All the extracts used in the present study lowered the blood glucose level in the diabetic treated rats. However, the extract from *T. crispa* stems showed the highest activity in lowering the blood glucose while the extract form *A. squamosa* leaves produced the lowest activity. The same results on lowering the blood glucose in diabetic rats were found when the extracts from *Tinospora crispa* stems ([12], [13] *Annona squamosa* leaves [13], *Pseuderanthemum palatiferum* leaves [14] and *Syzygium cumini* [15] were given orally to the diabetic rats.

Hematological values including RBC, WBC, Hct and Hb but not platelet, lymphocyte and monocyte were increased in the diabetic controls compared to normal controls. However, they were reversed in the diabetic rats treated with the extracts from *Tinospora crispa* stems, *Annona squamosa* leaves, *Musa sapientum* flowers, and *Piper sarmentosum* leaves. These data suggest that the extracts from *Tinospora crispa* stems, *Annona squamosa* leaves, *Musa sapientum* flowers, and *Piper sarmentosum* leaves can recover the pathology of the hematological values in the diabetic rats. Again, the results obtained by using these extracts are similar to using *Phellinus ignarius* (L) Quel [10] and *Musa sapientum* [16].

In line with the hematological values, cholesterol, triglyceride, and LDL were increased but not HDL was decreased in the diabetic controls. These values were reversed by the treatment of the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves. These evidences reveal that these extracts have hypolipidemic activity.

Decreased serum insulin found in the diabetic controls. However, it was increased in the diabetic rats treated with the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves. This result indicating the extracts have hyperinsulinemic activity.

Although, glucose was found in urine of the diabetic controls and diabetic treated rats, the other chemical properties of urine in normal controls, diabetic controls and diabetic treated rats were not different. This information revealing the extracts have no effect on renal function.

In conclusion, the extracts from *T. crispa* stems, *A. squamosa* leaves, *M. sapientum* flowers, and *P. sarmentosum* leaves have non-acute toxicity with LD_{50}>2000mg/kg and have hypoglycemic, hypolipidemic and hyperinsulinemic activities.

ACKNOWLEDGMENT

This work is partly supported by PMI 2 Connect-Research Co-operation Award. We greatly acknowledge the generous assistance from Prof. Anwar Baydoun and thank members of BSc and PhD students in the Department of Biology, Faculty of Science, Mahasarakham University, who have provided invaluable contribution to the work, in particular, Ms.Tippayaporn Poolsawat, Ms.Juthaporn Tongpon and Ms.Ratirose Wirat.

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