Mahidol University Journal of Pharmaceutical Sciences 2005; 32(1-2): 7-14.

Original Article

Acute and Subacute Toxicities of the Ethanol Extract from *Alternanthera philoxeroides* Griseb.

S. Thanabhorn,[1,2*] K. Jaijoy,[2] S. Thamaree,[3] K. Ingkaninan[4,5] and A. Panthong[6]

[1]Division of Pharmacology, Department of Preclinical Science, [2]Research Unit of Pharmacology and Toxicology, Research Center, Faculty of Medicine, Thammasat University, Pathum Thani, [3]Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, [4]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, [5]Cosmetic and Natural Product Research Center, The Institute of Health Science Research, Naresuan University, Phitsanulok, [6]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Abstract The toxicity studies were carried out in male and female rats with a 95% ethanol extract of *A. philoxeroides* Griseb. The oral acute toxicity test at 5,000 mg/kg revealed that the ethanol extract did not produce toxic effects on signs, general behaviors, mortality and gross appearance of internal organs of rats. In addition, the oral subacute toxicity studies in rats at the dose of 1,000 mg/kg/day displayed no significant changes in body and internal organs weights, hematological and clinical blood chemistry values. Pathological examination showed normal architecture of all internal organs. Thus, the ethanol extract of *A. philoxeroides* did not produce oral acute or subacute toxicity in rats.

Keywords: acute toxicity, *Alternanthera. philoxeroides*, subacute toxicity

INTRODUCTION

Alternanthera philoxeroides Griseb. (Amaranthaceae), Thai name "Phak Pet Nam" or "Phak Pet", is an emerged aquatic plant, but can grow in dry land. This plant has originated in South America and spread over many parts of the world. It is considered an invasive species in Australia, China, New Zealand, Thailand and the United States. The whole plant of *A. philoxeroides* is used in traditional medicine for the treatment of wound, fever and milk secretion.[1] Importantly, several studies have reported a potent antiviral activity of *A. philoxeroides* against epidemic hemorrhage fever virus (EHFV),[2,3] dengue virus,[4] influenza,[5] human herpes virus-6 (HHV-6),[6] and human immunodeficiency virus (HIV).[7] In addition, the whole plant has recently been verified for its HIV inhibition effect.[8] However, the toxicity of *A. philoxeroides* has not been intensively studied. This study was undertaken to contribute data on safety of the 95% ethanol extract from *A. philoxeroides* in rats by determining both oral acute and subacute toxicities.

MATERIALS AND METHODS

Plant Material

A. philoxeroides was collected from Phitsanulok, Thailand in April 2002. The plant materials were identified in the Pharmaceutical Botany Mahidol (PBM) Herbarium. The voucher specimen (Fansai 0005) was kept at Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok and deposited in the PBM Herbarium, Faculty of Pharmacy, Mahidol University, Bangkok.

[*]**Corresponding author:** Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand.
Fax: (662) 926-9711. Email: tseehanat@yahoo.com

Preparation of Plant Extract

The whole plant was cut into small pieces and dried in a hot air oven at 55ºC. The dried materials were ground and macerated in 95% ethanol for three days and filtered. The filtrate was evaporated under reduced pressure until dryness. The residue from the filtration was macerated in 95% ethanol again for three days, then filtered and evaporated. The final extract was combined with the first one and thin layer chromatography (TLC) fingerprints of the extract were recorded.

Laboratory Animals

Male and female Sprague-Dawley rats, weighing 120-160 g, were obtained from the National Laboratory Animal Center, Nakhon Pathom, Thailand. They were all clinically healthy and maintained in environmentally controlled conditions at 24 ± 1°C under a 12-hour dark-light cycle, and given a standard diet and water *ad libitum*, throughout the experimental period.

Acute Toxicity Study

According to the World Health Organization (WHO) guideline[9] and the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals,[10] ten rats of either sex were divided into two groups of five animals per sex. The 2,500 mg/ml ethanol extract in 10% dimethyl-sulfoxide (DMSO) was administered to rats at single oral dose of 5,000 mg/kg body weight by gavage, and 10% DMSO was also given to the control group of rats. All rats were observed at the first, second, fourth and sixth hours and once daily over 14 days for clinical signs of toxicity such as changes in rate and depth of breathing, color of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures, contraction of voluntary muscle, and loss of reflex, etc. The survival rats were weighed daily and observed further for clinical signs of toxicity for up to 14 days. After the experimental period, rats of both groups were sacrificed and their internal organs such as heart, lungs, livers, kidneys, spleen, adrenals, sex organs and brain were examined.

Subacute Toxicity Study

The method was performed following the protocol described by the WHO guideline[9] and the OECD guideline for testing of chemicals.[11] Unless the test substance at the dose of 5,000 mg/kg produced any signs of acute toxicity, a dose of 1,000 mg/kg was chosen in the subacute toxicity test for daily administration to rats for 14 days. Male and female rats were randomly divided into three groups of ten. The extract was orally given to a treated group at a dose of 1,000 mg/kg/day, while the control group received the vehicle at the same volume. The satellite group was orally treated with the extract at daily dose of 1,000 mg/kg/day for 14 days, and no further treatment for the following 14 days to determine the reversibility of toxic effects. Body weight, clinical signs of toxicity and mortality were monitored during the test period.

After the treatment period, all rats were fasted overnight and anesthetized with ether for blood collection. Blood was collected from the common carotid artery into a heparinized tube for hematological studies (complete blood count, red blood cell count, platelet count and red cell indices). The serum was separated for determining the concentrations of glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total bilirubin, direct bilirubin, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP).

After the blood collection, internal organs such as heart, lungs, thymus, livers, kidneys, spleen, adrenals, small intestine, stomach and duodenum, muscle with sciatic nerve, thoracic spines, brain, eyes, sex organs, uterus and epididymis were removed from all rats for detection of gross lesions. All tissues were fixed in 10% buffered formalin solution. After routine processing, the paraffin sections of each tissue were cut at 5 μm thickness and stained with haematoxylin and eosin for a microscopic examination.

Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. The data obtained from acute toxicity studies were analyzed using Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

After administration of a single oral dose of the 95% ethanol extract from *A. philoxeroides* at 5,000 mg/kg, neither sign of toxicity nor death among the rats was observed during 14 days of the acute toxicity experimental period. Toxicity evaluation was further carried out by observing body weight gain and internal organs' weights of the animals as summarized in Tables 1 and 2. As compared with the control group, the body weight and internal organ weights of treated rats showed no significant changes. Moreover, gross examinations of the internal organs revealed no pathological abnormalities relative to the control. These results suggest that the 95% ethanol extract from the whole plant of *A. philoxeroides* is practically not toxic after an acute exposure. In the subacute toxicity study at the dose of 1,000 mg/kg/day, the body weight and the body weight gain of animals were not significantly different in all the groups of both sex (Table 3). Furthermore, neither changes in animal behaviors, nor toxic signs were detected in the treated rats as compared to the control. Following necropsy, no macroscopic change in the internal organs of all rats was observed. As shown in Table 4, the weights of some internal organs were

Table 1. Body weights of rats in acute toxicity of the ethanol extract of *A. philoxeroides* (5,000 mg/kg)

	Body weight (g)			Weight gain (g) on day 14
	Day 0	Day 7	Day 14	
Female				
Control	132.00 ± 5.61	171.00 ± 4.00	180.40 ± 6.71	50.40 ± 2.73
A. philoxeroides	127.00 ± 4.36	166.00 ± 4.30	180.40 ± 6.43	53.40 ± 3.28
Male				
Control	130.00 ± 6.52	192.00 ± 2.00	220.20 ± 7.70	90.20 ± 5.36
A. philoxeroides	135.00 ± 2.24	190.00 ± 4.30	222.60 ± 5.65	87.60 ± 4.95

Values are expressed as mean ± S.E.M., n = 5. There were no significant differences at $p < 0.05$.

Table 2. Organ weights (in grams) of rats in acute toxicity of the ethanol extract of *A. philoxeroides* (5,000 mg/kg)

	Control	*A. philoxeroides*
Female		
Lung	0.99 ± 0.05	0.97 ± 0.03
Heart	0.76 ± 0.03	0.75 ± 0.03
Liver	7.57 ± 0.17	7.28 ± 0.40
Spleen	0.60 ± 0.04	0.66 ± 0.06
Adrenal	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.85 ± 0.01	0.84 ± 0.01
Ovary	0.05 ± 0.00	0.06 ± 0.00
Male		
Lung	1.02 ± 0.06	0.98 ± 0.03
Heart	0.88 ± 0.02	0.82 ± 0.04
Liver	10.10 ± 0.41	9.70 ± 0.40
Spleen	0.77 ± 0.04	0.74 ± 0.03
Adrenal	0.02 ± 0.00	0.02 ± 0.00
Kidney	1.14 ± 0.06	1.05 ± 0.03
Testis	1.22 ± 0.03	1.18 ± 0.04

Values are expressed as mean ± S.E.M., n = 5. There were no significant differences at $p < 0.05$.

Table 3. Body weights of rats in subacute toxicity of the ethanol extract of *A. philoxeroides*

	Body weight (g)			Weight gain (g)
	Day 0	Day 14	Day 28	on day 14
Female				
Control	147.00 ± 10.69	173.50 ± 8.88	-	25.25 ± 3.70
A. philoxeroides[a]	147.50 ± 11.49	179.25 ± 8.68	-	31.75 ± 4.83
A. philoxeroides[b]	148.00 ± 14.99	174.29 ± 12.39	204.80 ± 11.60	26.29 ± 3.39
Male				
Control	142.67 ± 6.64	210.00 ± 10.26	-	67.33 ± 5.36
A. philoxeroides[a]	149.75 ± 6.72	222.50 ± 5.05	-	72.75 ± 3.44
A. philoxeroides[b]	144.75 ± 9.20	216.50 ± 11.72	296.33 ± 7.27	71.75 ± 4.71

Values are expressed as mean ± S.E.M., n = 10. There were no significant differences at $p < 0.05$.
[a] A group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days.
[b] A satellite group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

Table 4. Organ weights (in grams) of rats in subacute toxicity of the ethanol extract of *A. philoxeroides*

	Control	*A. philoxeroides*[a]	*A. philoxeroides*[b]
Female			
Lung	1.09 ± 0.04	1.06 ± 0.03	1.22 ± 0.04*
Heart	0.81 ± 0.03	0.87 ± 0.02	0.89 ± 0.03
Liver	5.78 ± 0.22	6.17 ± 0.32	6.60 ± 0.32
Spleen	0.57 ± 0.02	0.62 ± 0.02	0.63 ± 0.03
Adrenal	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.84 ± 0.02	0.87 ± 0.03	0.92 ± 0.03*
Ovary	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01*
Male			
Lung	1.27 ± 0.03	1.45 ± 0.17	1.47 ± 0.12
Heart	1.16 ± 0.03	1.16 ± 0.04	1.16 ± 0.09
Liver	8.16 ± 0.41	8.03 ± 0.26	9.72 ± 0.75*
Spleen	0.73 ± 0.05	0.83 ± 0.02	0.80 ± 0.07
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
Kidney	1.10 ± 0.04	1.03 ± 0.02	1.20 ± 0.05
Testis	1.26 ± 0.03	1.39 ± 0.07	1.61 ± 0.05*

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days.
[b] A satellite group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days followed no treatment for 14 days. * Significantly different from control, $p < 0.05$.

significantly different between the treated and control groups. In the female satellite group, the weights of lung, kidney and ovary were slightly higher than those of the control. The weights of liver and testis of the male satellite group were slightly increased. Nonetheless, all of the increases were minute and the difference may be due to variation in the size of internal organs and/or body weight of rats.[12]

To evaluate intravascular effect and bone marrow activity in the rats treated with the ethanol extract, hematological parameters were examined as present in Tables 5 and 6. Since the significance of these hematological parameters perhaps represents pathological conditions especially anemia in which the mean corpuscular hemoglobin (MCH), the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) values would decrease. However, not only those values but the morphology of red blood from blood smear examination is also important to validate the anemia condition. The MCV of the extract-treated male group and the male satellite group was somewhat

decreased as compared to the control. The MCHC of the male satellite group was increased to a certain extent. Nonetheless, the changes of MCV and MCHC remained within the normal ranges[13,14] and the blood smear results revealed normal characteristics (data not shown). To evaluate the sign of internal organ damages, clinical blood chemistry examination was performed and the results were shown in Table 7. High serum creatinine may result from the pre-renal factors (congestive heart failure, shock and depletion of sodium and water), renal factors (the abnormalities in glomeruli, tubules,

Table 5. Hematological values of rats in subacute toxicity of the ethanol extract of *A. philoxeroides*

	Control	*A. philoxeroides*[a]	*A. philoxeroides*[b]
Female			
Red blood cells ($x10^6$/μl)	8.08 ± 0.11	8.19 ± 0.23	7.88 ± 0.31
Hemoglobin (g/dl)	14.50 ± 0.63	15.38 ± 0.31	14.84 ± 0.64
Hematocrit (%)	43.20 ± 1.85	46.20 ± 2.06	43.60 ± 1.72
Mean corpuscular volume (fl)	57.08 ± 0.29	57.02 ± 0.72	55.44 ± 0.70
Mean corpuscular hemoglobin (pg)	19.28 ± 0.21	19.02 ± 0.30	18.78 ± 0.17
Mean corpuscular hemoglobin concentration (g/dl)	33.54 ± 0.35	33.40 ± 0.56	33.88 ± 0.43
Platelet ($x10^5$/μl)	7.50 ± 0.26	6.99 ± 0.73	6.61 ± 0.96
Male			
Red blood cells ($x10^6$/μl)	7.78 ± 0.21	7.83 ± 0.17	7.82 ± 0.16
Hemoglobin (g/dl)	14.46 ± 0.50	14.94 ± 0.29	14.94 ± 0.48
Hematocrit (%)	46.43 ± 3.99	46.86 ± 2.34	45.43 ± 1.04
Mean corpuscular volume (fl)	61.04 ± 0.24	60.11 ± 0.37*	57.93 ± 0.25*
Mean corpuscular hemoglobin (pg)	19.11 ± 0.15	19.12 ± 0.25	19.07 ± 0.24
Mean corpuscular hemoglobin concentration (g/dl)	31.81 ± 0.23	32.08 ± 0.36	32.93 ± 0.46*
Platelet ($x10^5$/μl)	8.18 ± 0.62	9.76 ± 0.88	8.18 ± 0.62

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days.
[b] A satellite group was given the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days. * Significantly different from control, $p < 0.05$.

Table 6. Differential white blood cell count values of rats in subacute toxicity of the ethanol extract of *A. philoxeroides*

	Control	*A. philoxeroides*[a]	*A. philoxeroides*[b]
Female			
White blood cell ($x10^3$/μl)	2.12 ± 0.43	2.59 ± 0.80	2.24 ± 0.43
Neutrophil (%)	15.80 ± 0.86	15.00 ± 3.70	15.00 ± 6.04
Lymphocyte (%)	82.20 ± 0.80	68.00 ± 4.86	72.20 ± 7.07
Monocyte (%)	1.40 ± 0.75	1.40 ± 1.17	1.40 ± 1.33
Eosinophil (%)	3.40 ± 0.51	3.60 ± 0.51	3.80 ± 2.92
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Male			
White blood cell ($x10^3$/μl)	2.97 ± 0.25	2.78 ± 0.25	3.22 ± 0.36
Neutrophil (%)	15.42 ± 2.18	18.00 ± 2.03	13.42 ± 2.74
Lymphocyte (%)	76.43 ± 2.88	77.71 ± 2.00	84.28 ± 3.04
Monocyte (%)	1.43 ± 0.37	2.43 ± 0.68	0.43 ± 0.20
Eosinophil (%)	1.00 ± 0.38	1.71 ± 0.36	1.71 ± 0.28
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days.
[b] A satellite group was given the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days. There were no significant differences at $p < 0.05$.

Table 7. Clinical blood chemistry values of rats in subacute toxicity of the ethanol extract of *A. philoxeroides*

	Control	*A. philoxeroides*[a]	*A. philoxeroides*[b]
Female			
Glucose (mg/dl)	103.80 ± 3.64	116.60 ± 4.80	111.80 ± 12.08
BUN (mg/dl)	19.80 ± 0.58	20.20 ± 0.58	21.40 ± 1.91
Creatinine (mg/dl)	0.37 ± 0.02	0.38 ± 0.02	0.36 ± 0.02
Total protein (g/dl)	5.26 ± 0.05	5.16 ± 0.07	5.16 ± 0.12
Albumin (g/dl)	2.64 ± 0.05	2.70 ± 0.04	2.64 ± 0.09
Total bilirubin (mg/dl)	0.26 ± 0.09	0.27 ± 0.02	0.32 ± 0.33
Direct bilirubin (mg/dl)	0.22 ± 0.10	0.18 ± 0.03	0.32 ± 0.36
SGOT (U/L)	128.00 ± 17.20	134.60 ± 15.05	117.00 ± 16.85
SGPT (U/L)	33.20 ± 1.80	32.60 ± 1.03	35.40 ± 1.72
ALP (U/L)	100.00 ± 8.09	96.00 ± 13.64	83.40 ± 8.98
Male			
Glucose (mg/dl)	106.14 ± 2.30	99.43 ± 4.44	105.86 ± 8.97
BUN (mg/dl)	18.29 ± 4.18	14.86 ± 0.70	19.57 ± 2.16
Creatinine (mg/dl)	0.36 ± 0.02	0.39 ± 0.01	0.43 ± 0.02*
Total protein (g/dl)	4.90 ± 0.10	5.06 ± 0.08	4.99 ± 0.13
Albumin (g/dl)	2.34 ± 0.08	2.39 ± 0.08	2.51 ± 0.08
Total bilirubin (mg/dl)	0.19 ± 0.03	0.24 ± 0.03	0.23 ± 0.06
Direct bilirubin (mg/dl)	0.15 ± 0.04	0.10 ± 0.04	0.14 ± 0.05
SGOT (U/L)	115.86 ± 13.56	110.71 ± 14.26	114.29 ± 13.00
SGPT (U/L)	35.43 ± 0.90	34.86 ± 1.20	42.86 ± 3.17*
ALP (U/L)	153.43 ± 5.70	152.29 ± 5.20	127.71 ± 7.83*

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was treated with the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days.
[b] A satellite group was given the extract of *A. philoxeroides* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days. * Significantly different from control, $p < 0.05$.

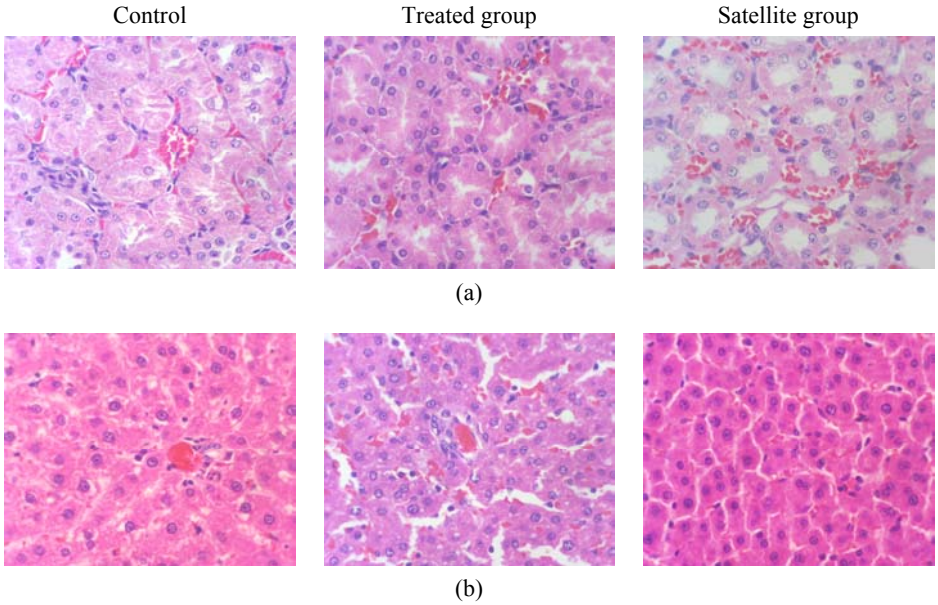

Figure 1. The histology of kidney (a) and liver (b). No significant damage was detected in any treatment group.

interstitial tissues or blood vessels of kidney) and post-renal factors (benign prostatic hypertrophy, ureter abnormalities and stone). The hepatic cell damage and the cholestasis of liver would show an increase in enzymes SGPT and alkaline phosphatase, respectively. The increased levels of those enzymes more than one fold would then be highly significant for clinical pathology with abnormalities in physical appearances. In male male satellite group, the administration of the ethanol extract of *A. philoxeroides* markedly caused an increase in the concentrations of creatinine and SGPT and a decrease of alkaline phosphatase as compared to those of the control group. However, the observed difference was less than one fold and no physical abnormalities of the animals were detected. Furthermore, the animals in the satellite groups were older than the control, which then account for the statistical variation in the above values from that of the control group. Still, the levels of these clinical blood chemical parameters were within the normal ranges.[13,15-18] Because the clinical blood chemical parameters were the indices of kidney, liver and pancreas functions, it is reasonable to conclude that the ethanol extract did not induce any lesions to the liver, kidney and pancreas. These observations were further confirmed by the histological assessment of the organs. The results showed that the ethanol extract of *A. philoxeroides* did not produce a significant damage in the vital organs (Figure 1) and the other internal organs (data not shown).

In conclusion, the 95% ethanol extract from the whole plant of *A. philoxeroides* did not cause oral acute or subacute toxicity in rats. Further study is in progress in order to evaluate the chronic toxicity for the safe use of this plant.

ACKNOWLEDGEMENTS

The authors are grateful to the National Research Council of Thailand for financial support and to Associate Professor Dr. Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, for the plant material identification

REFERENCES

1. Thiengburanathum W. Dictionary of Thai Herbs. Bangkok: O.S. Printing House, 2531.
2. Yang ZQ, Zhang MY, Liu JJ, *et al.* Extraction of effective parts of *Alternanthera philoxeroids* (Mavt.) Griseb. and its antiviral effect. *Zhongguo Zhong Yao Za Zhi* 1989; 14: 488-90.
3. Qu CF, Yang ZQ, Xiang JM. *Alternanthera philoxeroids* (Mavt.) Griseb. protection against fetal epidemic hemorrhagic fever virus infection in suckling mice. *Zhongguo Zhong Yao Za Zhi* 1993; 18: 304-5.
4. Jiang WL, Luo XL, Kuang SL. Effects of *Alternanthera philoxeroids* Griseb. against dengue virus *in vitro*. *Di Yi Jun Yi Da Xue Xue Bao* 2005; 25: 454-6.
5. Niu RJ. A study of the preventive and therapeutic effects of *Alternathera philoxeroides* on influenza. *Zhong Xi Yi Jie he Za Zhi* 1986; 6: 29-30 [Article in Chinese].
6. Hui-wu Y, Xin-quan Z, Dong-ping L, *et al*. Antiviral activity of CH_{31} against HHV-6 *in vitro*. *Antiviral Res* 1995; 26: A359.
7. Zhang SM, He YS, Tabba HD, *et al*. Inhibitor against the human immunodeficiency virus in aqueous extract of *Alternanthera philoxeroids*. *Chin Med J* (Engl) 1988; 101: 861-6.
8. Lousirirojanakul S, Chaiprasert U, Thamaree S, *et al*. A study of anti HIV, antifungal, antimicrobial activities and immunomodulating activities of *Antidesma acidum* Retz., Euphobiaceae and four other herbs. A report submitted to the National Research Council of Thailand, 2003.
9. World Health Organization (WHO). General guidelines for methodologies on research and evaluation of traditional medicine, Switzerand, 2000.
10. The Organization of Economic Co-operation and Development (OECD). The OECD guideline for testing of chemical: 420 Acute oral toxicity, France, 2001.
11. The Organization of Economic Co-operation and Development (OECD). The OECD guideline for testing of chemical: 407 Repeated dose oral toxicity - rodent: 28-day or 14-day study, France, 1981.
12. Auletta CS. Acute, subchronic and chronic toxicology. In: Derelanko MJ, Hollinger MA, eds. Boca Raton: CRC Press, 1995: 51-104.

13. Levine BS. Animal clinical pathology. In: Derelanko MJ, Hollinger MA, eds. Boca Raton: CRC Press, 1995: 517-37.
14. Feldman BF, Zinkl JG, Jain NC, *et al*. Schalm's Veterinary Hematology, 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000.
15. Vondruska JF, Greco RA. Certain hematological and blood chemical values in Charles River CD albino rats. *Bull Am Soc Vet Clin Pathol* 1973; 2: 3-17.
16. Caisey JD, King DJ. Clinical chemical values for some common laboratory animals. *Clin Chem* 1980; 26: 1877-9.
17. Sacher RA, McPherson RA. General chemistry. In: Widmann's Clinical Interpretation of Laboratory Test, 10th ed. Philadelphia: Davis Company, 1991: 318-65.
18. Sacher RA, McPherson RA. Test of liver function. In: Widmann's Clinical Interpretation of Laboratory Test, 10th ed. Philadelphia: Davis Company, 1991: 416-43.