The dried *Sophora alopecuroides* seed was ground to a fine powder with a grinder and passed through a 40-mesh sieve, extracted with chloroform, 95% ethanol and distilled water. The extracts were lyophilized, and the yields obtained are given in Table 1. The chemical compounds of *Sophora alopecuroides* seed were separated by thin layer chromatography, and the kinds of the compounds present in the extract were determined with the use of spray reagents (Table 4.2, Table 4.3, and Table 4.4). These reagents are very useful because they can confirm the presence of a particular compound by turning a specific color. The TLC revealed that the chloroform extract of *Sophora alopecuroides* seed positive for the presence of alkaloids as determined by the development of orange color with Dragendroff’s reagent and saponins by blue color with anisaldehyde-H$_2$SO$_4$ reagent (Table 4.2). Saponins were also detected in the 95% ethanol extract (Table 4.3). Flavonoids were detected in the 95% ethanol extract (Table 4.3) and distilled water extract (Table 4.4) by the development of yellow color with anisaldehyde-H$_2$SO$_4$ reagent, but were not detected in the chloroform extract, probably because they were present in very low concentrations. Terpenoids were detected only in the 95% ethanol extract by the development of purple color with vanillin-H$_2$SO$_4$ reagent (Table 4.3). The color tests strongly suggest that alkaloids were present in all the extracts of *Sophora alopecuroides* seed. Alkaloids are a group of naturally occurring chemical compound that contain basic nitrogen atoms. This distinction can easily be seen based on reaction of alkaloid compounds with potassium iodide in Dragendroff’s reagent. The total alkaloids content was analyzed and found 7.56% in dried sample. The yield of chloroform extract (Table 1) was found 3.39% of dried sample.
The alkaloids present were identified with Q-TQF MS. In Table 4.5 showed that nine alkaloid have been identified namely sophocarpine, matrine, baptifoline, oxysophocarpine, oxymatrine, sophocarpine dimer, oxysophocarpine dimer, oxymatrine dimer and sophoranol-N-oxide dimer in the seed extract of *Sophora alopecuroides*. In this study, direct infusion mass spectrometry analysis was used to determine the alkaloid profile in the 95% ethanol and distilled water extracts of *S. alopecuroides* seed. It was found that all two extracts exhibited similar spectrum as depicted in Figure 4.1. Data (pseudo molecular ion, collision energy and main fragment ions observed in MS²) for compounds detected in all extracts along with their corresponding reported values for comparison are summarized in Table 4.5. The molecular weight and fragment ions of compound no 1, 2, 4, 5, 6, 7, 8, and 9 were in agreement with those reported in the literature, in which the fragmentation pattern of each compounds have been well described (Guo, *et al.*, 2011).

The DPPH scavenging activity assay was carried out to evaluate the ability of antioxidants to scavenge free radicals. In this assay the violet color of DPPH was changed to a pale yellow color because of the abstraction of hydrogen atom from the antioxidant compound. The more antioxidant compound in the extract, the more the DPPH reduction will occur. More complete reduction of DPPH is related to the high scavenging activity performed by particular compounds. The alkaloids possessed hydroxyl group and this indicated that the alkaloids have the potential to be good antioxidant agent. The alkaloids in Sophora species contain hydroxyl group such as hydroxymatrine and oxysophocarpine have been reported in the early study (Xiu, *et al.*, 2010). In this study, the scavenging activity of *Sophora alopecuroides* seed extracts was compared with ascorbic acid which is well known natural antioxidants. As shown in Figure 4.2, the scavenging activity of *Sophora alopecuroides* seed are almost 40 fold lower than that of ascorbic acid (IC₅₀ = 3.69 ± 0.01 µg/ml). The IC₅₀ value of ethanol
extract was found to be 155.33 ± 0.06 µg/ml and aqueous extract was 167.47 ± 0.03 µg/ml. The scavenging activity of *Sophora alopecuroides* seed increased with the increasing concentrations, and the both of the extracts shown almost the same activities. This may be due to the both of extracts contain similar compounds and its concentrations are almost the same.

The ferric reducing antioxidant power (FRAP) assay was carried out to evaluate the ability of *Sophora alopecuroides* seed extract to reduce the ferum ion in relation to its antioxidant activity. The ferric reducing activity of were analyzed based on the reduction of ferric-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) to blue ferrous-TPTZ. As shown in Figure 4.3, the extracts of *Sophora alopecuroides* seed gave significant reducing power activities when compared to standard ascorbic acid (IC\(_{50}\) = 5.37 ± 0.03 µg/ml) and its activities were increased with the increasing concentration of sample. The IC\(_{50}\) value found for ethanol extract was 9.71 ± 0.02 µg/ml and water extract was 10.10 ± 0.02 µg/ml respectively.

Enzyme reactions are typically studied under steady state conditions since under these conditions the reaction velocity remains constant. The crude extracts of *Sophora alopecuroides* seed were tested for inhibition of GPa under steady state conditions in a spectrophotometric assay that is detailed in Materials and Methods. Glycogen phosphorylase-a from rabbit muscle was used because of the expense of the human enzyme. Glucose 1-phosphate was the substrate used and it can be degraded by GPa to release phosphate, which has intensive green color when react with malachite green that absorbs light at 620 nm (Figure 4.4). Therefore, enzyme activity is directly proportional to the absorbance at 620 nm. The effect on GPa activity by any sample can easily be determined by comparing the absorbance at 620 nm to the control.
The assay was carried out at pH 7.2 because the optimal pH for GPa activity was between 6.8 and 8.2 (Loughlin, et al., 2008). The enzyme and substrate were both dissolved in 50 mM Hepes buffer. The extracts were dissolved in 10% DMSO in 50 mM Hepes buffer. Appropriate controls and blanks were used. The control consisted of the solution that dissolved sample. Blanks were especially required because the extracts were colored. To account for this, the blanks contained buffer and extract. The extracts were tested for inhibition of GPa at different concentrations (2-32 mg/l). The results (Figure 4.4) show that aqueous extract exhibited little or no inhibition (< 20%) at the concentration tested and no more difference in inhibition has been observed. The standard drug, caffeine, gave 71.52% inhibition and its IC$_{50}$ was 620.40 ± 0.01 µg/ml. The ethanol extract showed 77.52% inhibition at 32 mg/ml under the assay conditions and its IC$_{50}$ was determined to be 581.61 ± 0.02 µg/ml. The ethanol extract of *Sophora alopecuroides* seed was the most active in inhibition of GPa, and such inhibitors are expected to be useful for treatment of diabetic. Therefore, it was selected for evaluation of its antihyperglycemic activity in diabetic rats.

In the present studies, acute toxicity test revealed that toxic reaction was found in non-diabetic rats when the ethanol extract of *Sophora alopecuroides* seed was administered to the rats at the dose of 5 mg/kg. The toxicity was observed by death of two female rats during 15 days of study period. This may be due to the possibility of low toxic alkaloid such as matrine (Dai, et al., 2009) is present in *Sophora alopecuroides* seed (Table 4.5). No toxic sign such as restless, response to touch, fearfulness, urination, or death was found in male rats at any of the doses given to each rat until the end of study. This may be to the individual differences of rodents to the toxic nature. In OGTT, Glucose administration to non-diabetic rats fasted for 16 hours increased plasma glucose levels from 4.68 ± 0.19 to 8.15 ± 0.61 mmol at 30 minutes and returned to normal at 2 hours. Administration of *Sophora alopecuroides* seed
ethanol extract to glucose loaded non-diabetic rats at the dose of 500 mg/kg body weight showed significant decrease in plasma glucose levels at 30 min, and no significant differences was observed treatment with the extract at the dose of 250 mg/kg body weight compared to the normal group (Figure 4.5, *P < 0.05; control vs. SAS Ext 500 mg/kg). From this result it indicates that *Sophora alopecuroides* seed extract (500 mg/kg) can improve glucose tolerance in non-diabetic rats.

STZ causes hyperglycemia by selective destruction of β cells in the islets of Langerhans (Sancheti, *et al.*, 2010), and nicotinamide protects β cells caused by STZ toxicity (Huang, *et al.*, 2010). Rats were induced diabetic by injection of STZ and nicotinamide mimicking the picture of type 2 diabetes which is most common to humans (Huang, *et al.*, 2010). In this experiment, antihyperglycemic activity of the extract was evaluated streptozotocin (STZ)-nicotinamide induced diabetic rats. The diabetic rats were confirmed by the presence of high fasting plasma glucose level which is the characteristic features of diabetic mellitus. The results (Table 4.6) showed that fasting blood glucose levels were significantly increased in STZ treated group compared to the normal control group (*P < 0.05; diabetic control vs. normal control). This is due to the lack of insulin in STZ-nicotinamide induced rats by destructing the β cells which leads to hyperglycemia. Treatment of these diabetic rats with *Sophora alopecuroides* seed extract (500mg/kg) decreased fasting plasma glucose levels significantly (*P < 0.05) on the 28th day by 43.7% compared to the 0th day. Another group treated with SAS at the dose of 250 mg/kg body weight did not show significant (*P > 0.05) glucose lowering effect at the end of experiment compared to the 0th day, but it decreased glucose level on the 28th day by 18.7%. Glucose lowering activity of the extract (250 mg/kg) was remained statistically significant compared to the diabetic control. From this result it indicates that SAS can decrease fasting plasma glucose level in a dose-dependent manner in STZ-nicotinamide induced diabetic rats. The diabetic rats treated
with standard drug glibenclamide showed significant reduction in fasting plasma glucose levels compared to diabetic control group (*P < 0.05; diabetic control vs. group III), and the same effect was observed SAS (500 mg/kg) treated group. It is well known that sulfonylurea causes hypoglycemia by increasing insulin secretion from pancreas (Del Prato and Pulizzi, 2006). The results in Table 4.6 showed that SAS extract in both of the doses (250 mg/kg and 500 mg/kg) produced hypoglycemia in diabetic rats. This strongly suggests that the possible mechanism by which the antihyperglycemic activity of *Sophora alopecuroides* seed due to its enhanced insulin secretion effect from pancreatic β cells like sulfonylurea. The reducing effect of *Sophora alopecuroides* seed extract could also be due to the action of alkaloid presence in SAS. The mechanism of its action is still unknown.

Weight loss is one of the major complications in diabetes and it arises due to the impairment in insulin action caused by STZ toxicity (Sancheti, *et al.*, 2010). Due to this there is a decrease in the body weight of STZ induced diabetic animals. As shown in Figure 4.6, treatment with STZ caused a significant (*P < 0.05) weight loss on the 28th day compared with normal control. This may be due to the reduction of insulin release from pancreatic β cells which leads to hyperglycemia, as a result increase muscle wasting and loss of tissue protein in diabetic rats (Salahuddin and Jalalpure, 2010). In contrast, rats in normal control group continued to gain weight (51.8%) during the 4-week of experimental period. Treatment with standard drug glibenclamide increased body weight by 6.1% in the first week, and declined again to nearly initial body weight in diabetic rats. Diabetic rats treated with SAS extract (250 mg/kg and 500 mg/kg) reduced body weight around 9% on the 28th day compared with their initial level. The levels of serum lipids are usually altered in diabetic mellitus (Shirwaikar, *et al.*, 2006). This was observed in diabetic rats in this study, where serum triglycerides and total cholesterol levels were significantly (*P < 0.05) elevated, and serum HDL cholesterol
levels were decreased 8.7% in comparison with normal control (Table 4.7). Treatment with *Sophora alopecuroides* seed extracts at the dose of 250 mg/kg and 500 mg/kg for 28 days to the diabetic rats significantly (*P < 0.05*) decreased serum triglycerides and total cholesterol levels compared to the diabetic control. Treatment with reference drug glibenclamide significantly decreased triglyceride, but there was no significant decrease in serum total cholesterol levels compared to diabetic control. The serum HDL levels in both of the SAS and glibenclamide treated groups were restored to the control level (Table 4.7). These data consistent with previous report which was saying that administration of high-fat diet with powdered fruit of *Sophora species* significantly decreased body weight in non-diabetic mice, exhibited lowering triglyceride and cholesterol effects while at the same time increasing HDL cholesterol in hyperlipidemic and cholesterol-fed rats (Hyun, *et al.*, 2008; Park, *et al.*, 2009). In summary, the present study showed that the administration of *Sophora alopecuroides* seed ethanol extract reduced blood glucose levels, body weight gain, and improved serum lipid profiles in diabetic rats. These results provide evidence that *Sophora alopecuroides* seed and its constituents are useful for the control of body weight and preventing diabetes related metabolic diseases.
Sophora alopecuroides seed (SAS) was extracted with chloroform, 95% ethanol and distilled water. All of the extracts showed alkaloid presence, and total alkaloid content was determined 7.56%.

Antioxidant activity of SAS extracts were evaluated by two in vitro bioassays: ferric reducing antioxidant power (FRAP) and scavenging of DPPH free radical assay. In FRAP assay, IC$_{50}$ value of ethanol extract was 9.71µg/ml, and IC$_{50}$ value of water extract was 10.10 µg/ml. Both of extracts showed less scavenging activity on DPPH free radical compared to standard ascorbic acid.

The crude ethanol and water extracts of Sophora alopecuroides seed were tested for inhibition of glycogen phosphorylase-a enzyme in an in vitro assay. The ethanol extract of Sophora alopecuroides seed showed the most potent inhibition of GPa enzyme and determined to have an IC$_{50}$ = 581.61 µg/ml, however water extract was inactive.

Acute toxicity of SAS ethanol extract was tested at increasing dose level in non-diabetic rats and the toxic effects has been observed at a dose of 5 g/kg body weight.

The ethanol extract of Sophora alopecuroides seed significantly improved glucose tolerance at the dose of 500 mg/kg body weight and no significant difference was observed at the dose of 250 mg/kg body weight compared to control.

Treatments with ethanol extract of Sophora alopecuroides seed for 28 days significantly decreased plasma glucose levels at the dose of 500 mg/kg body weight and it is ineffective at the dose of 250 mg/kg body weight compared to 0 day. But glucose lowering activity of the extract (250 mg/kg) was remained significant compared to the
diabetic control on the 28th day. The results showed that SAS extract in both of the
doses (250 mg/kg and 500 mg/kg) produced hypoglycemia in diabetic rats. This
suggests that the possible mechanism by which the antihyperglycemic activity of
Sophora alopecuroides seed depends on its enhanced insulin secretion effect from
pancreatic β cells like sulfonylurea. It is generally believed that herbal drugs operated
by a number of mechanisms to elicit their hypoglycemic effects. This experiment has
shown that the crude extract of SAS possessed GPa enzyme inhibitor. Therefore, the
antidiabetic effect of Sophora alopecuroides seed may have a multiply mechanism of
action, combining the effects of sulfonylurea and GPa inhibitor.

Diabetic rats treated with SAS extract (250 mg/kg and 500 mg/kg) reduced body
weight around 9% on the 28th day compared with their initial levels, significantly (*P <
0.05) decreased serum triglycerides and total cholesterol levels, and the serum HDL
levels were increased compared to the diabetic control.

The results obtained from this study provide scientific evidence which is
supporting of the traditional use of Sophora alopecuroides seed as an antidiabetic
remedy and its complications.