

Full Length Research Paper

## Hypoglycemic effect of *Lagerstroemia speciosa* (L.) Pers. on alloxan-induced diabetic mice

N. C. Tanquilut<sup>1</sup>, M. R. C. Tanquilut<sup>2</sup>, M. A. C. Estacio<sup>3</sup>, E. B. Torres<sup>3</sup>, J. C. Rosario<sup>4</sup> and B. A. S. Reyes<sup>1, 4\*</sup>

<sup>1</sup>Institute of Veterinary Medicine and Zootechnics, Pampanga Agricultural College, Magalang 2011, Pampanga, Philippines.

<sup>2</sup>Institute of Engineering and Computer Studies, Pampanga Agricultural College Magalang 2011, Pampanga, Philippines.

<sup>3</sup>College of Veterinary Medicine, University of the Philippines, Los Banos, Laguna, Philippines.

<sup>4</sup>Thomas Jefferson University, Department of Neurosurgery, Farber Institute for Neurosciences, Philadelphia, Pennsylvania 19107, USA.

Accepted 7 November, 2009

*Lagerstroemia speciosa* (L.) Pers. known as “banaba” is traditionally used as a herbal medicine in the Philippines. Although *Lagerstroemia speciosa* has been shown to produce hypoglycemic effects in some mice models of diabetes, there are no reports of the effects of this substance in alloxan-induced diabetic mice. Thus, the present study aimed to elucidate the hypoglycemic effects of *L. speciosa* in ICR strain of mice. Diabetes was induced by the intraperitoneal injection of alloxan. Spray dried *L. speciosa* powder (1000 mg/kg) or decoction (20 ml/kg) was administered on alloxan-induced diabetic male ICR mice for 28 days by gavage. The effects of *L. speciosa* on blood and urinary glucose levels and body weight, feed intake and water intake were measured. Spray dried *L. speciosa* powder and decoction significantly reduced blood ( $P < 0.01$ ) and urinary glucose ( $P < 0.05$ ) levels of diabetic mice from day 8 to 28 compared with the diabetic control. These mice also had lower ( $P < 0.05$ ) body weight compared with the diabetic control from day 15 to day 28. The feed intake of diabetic mice was higher ( $P < 0.05$ ) compared with non-diabetic control and *L. speciosa*-treated diabetic mice from day 22 to 28. A comparable fluid intake was evident among non-diabetic mice and *L. speciosa*-treated diabetic mice from day 8 to 28 which was significantly lower ( $P < 0.01$ ) compared with the diabetic mice. These results suggest that *L. speciosa* possesses beneficial antihyperglycemic activity in controlling the elevated glucose level in alloxan-induced diabetic mice.

**Key words:** Antihyperglycemic activity, *Lagerstroemia speciosa*, diabetes mellitus, alloxan-induced diabetes, mice.

### INTRODUCTION

*Lagerstroemia speciosa* L. Pers. (Lythraceae), commonly known as “banaba” in the Philippines is a tropical flowering tree used as a folk medicine for the treatment of a myriad of diseases (de Padua et al., 1997). Growing up to 30 m high with a 9 to 12 m spread, *L. speciosa* is regularly sold in local markets and herb health stores with its various plant parts (bark, leaves and flowers). It is popular as an anti-diabetic, diuretic, febrifuge, stimulant

and purgative (de Padua et al., 1997; Fernando et al., 2004). In addition, decoction of the seeds has been reported to demonstrate narcotic-like potential (de Padua et al., 1997; Fernando et al., 2004).

In the Philippines, the tea made from the leaves of *L. speciosa* has been used as a beverage for the treatment and prevention of diabetes mellitus (Quisumbing, 1978). Using a mouse model of type 2 diabetes (KK-A<sup>y</sup>/Ta Jelf), diet-containing extract from the leaves significantly suppressed the elevation of plasma glucose levels compared to the control mice fed with cellulose (Kakuda et al., 1996). It has been demonstrated that the extract possesses an anti-adipogenic activity by reducing weight

\*Corresponding author. E-mail: [bsr103@jefferson.edu](mailto:bsr103@jefferson.edu). Tel: (215) 503-5870. Fax: (215) 503-9238.

gain on parametrial adipose tissue in female diabetic KK-A<sup>y</sup> mice (Suzuki et al., 1999). *In vitro* studies using 3T3-L1 showed that the extract exhibits activities that stimulated both glucose transport and inhibited adipocyte differentiation (Liu et al., 2001; Bai et al., 2008). In rat adipocyte, leaves of *L. speciosa* increased the rate of glucose uptake and decreased the isoproterenol-induced glycerol release (Hayashi et al., 2002). Also, using Chinese hamster ovary cells expressing human insulin receptor, *L. speciosa* remarkably increased the Erk activity (Hattori et al., 2003).

Clinical use of *L. speciosa* in diabetic patients has been shown to suppress glucose level in a dose-dependent manner (Judy et al., 2003). While the hypoglycemic activity of *L. speciosa* extract has been tested in genetically diabetic KK-A<sup>y</sup> (Kakuda et al., 1996) and dd<sup>y</sup> (Takagi et al., 2008) mouse models of diabetes, to date, there are no reports of its effects on alloxan-induced diabetic mice. Moreover, most recent reports have demonstrated potential antidiabetic activity of *L. speciosa* *in vitro* (Klein et al., 2007; Hou et al., 2009). Thus, the present study investigated the anti-diabetic potential of *L. speciosa* in alloxan-induced diabetic mice.

*In vivo* and *in vitro* studies showed that preparations of *L. speciosa* influence bioavailability, thereby affecting their hypoglycemic potencies (Judy et al., 2003; Kakuda et al., 1996). Spray dried pharmaceutical products are gaining popularity because they are easily processed and packaged for clinical trials and commercialization (Toledo, 1980). Leaves of *L. speciosa* are traditionally prepared in a form of decoction (de Padua et al., 1997). Hence, this study also aimed to investigate the antidiabetic potencies of spray dried preparation and decoction in alloxan-induced diabetic mice.

## MATERIALS AND METHODS

Leaves of *L. speciosa* were collected from the University of the Philippines, Los Baños, College, Laguna, Philippines. Plant materials were identified and authenticated at the Botanical Herbarium, Museum of Natural History, from said University. The decoction was obtained by boiling 10 g of fresh mature chopped *L. speciosa* leaves in 100 ml of water for 5 min (Reyes et al., 2006) in a closed vessel and filtered. Spray dried powder was prepared following the same preparation for decoction. The leaves were thenosterized, filtered and the solution was powderized using a spray dryer (Niro Mobile<sup>R</sup> minor, Soebord, Denmark) at the Food Engineering Laboratory, Food Science Cluster, College of Agriculture, University of the Philippines, Los Banos, College, Laguna, Philippines. Maltodextrin (Maltrin, Muscatine, Iowa) served as carrier to prevent sticking of extract to the spray drying machine collection chamber. The decoction and spray dried powder were obtained from the same homogenate.

### Animals

Male ICR strain mice (Research Institute of Tropical Medicine, Department of Health, Alabang, Muntinlupa, Metro Manila, Philippines) weighing 25 - 40 g were caged individually. Food and water were given *ad libitum*. Mice were maintained on normal

laboratory chow diet.

### Induction of diabetes

Alloxan monohydrate (Sigma Chemical, St. Louis, OH) was injected intraperitoneally at a dose of 200 mg/kg body weight (BW). The induction of alloxan-induced diabetes was confirmed by measuring the blood glucose level. Mice with glucose levels above 200 mg/dl were subjected to further evaluation with a glucose challenge and a standardized oral glucose tolerance test to confirm diabetes mellitus. For proper evaluation of the test, the mice were deprived of food for 16 h. A zero time (baseline) blood glucose sample was drawn. The mice were then given oral glucose (2 g/kg) and the blood drawn at 30, 60 and 120 min intervals for measurement of glucose levels. One group of mice did not receive any substance and served as the non-diabetic control (n = 10). The diabetic mice were randomly divided into three groups consisting of 8 - 11 animals each. The mice in the first group served as the diabetic control. The second and third groups were given spray dried powder at 1,000 mg/kg and decoction at 20 ml/kg, respectively. Spray dried powder and decoction were administered via gavage. Blood samples were collected from the tail vein and measured with a glucometer (OneTouch<sup>R</sup> Ultra<sup>R</sup>, LifeScan, Inc., Milpitas, CA) on day 1 (D1), day 8 (D8), day 15 (D15), day 22 (D22) and day 29 (D29). Urinary glucose was also obtained using urine test strips on days 1 (D1), 5 (D5), 9 (D9), 13 (D13), 17 (D17), 21 (D21), 25 (D25) and 29 (D29). Urinary glucose was tested more often than the blood glucose level since it is easier to sample and less stressful to the mice while providing confirmation of the trend of the blood glucose levels. Daily body weight, feed intake and liquid intake were also measured.

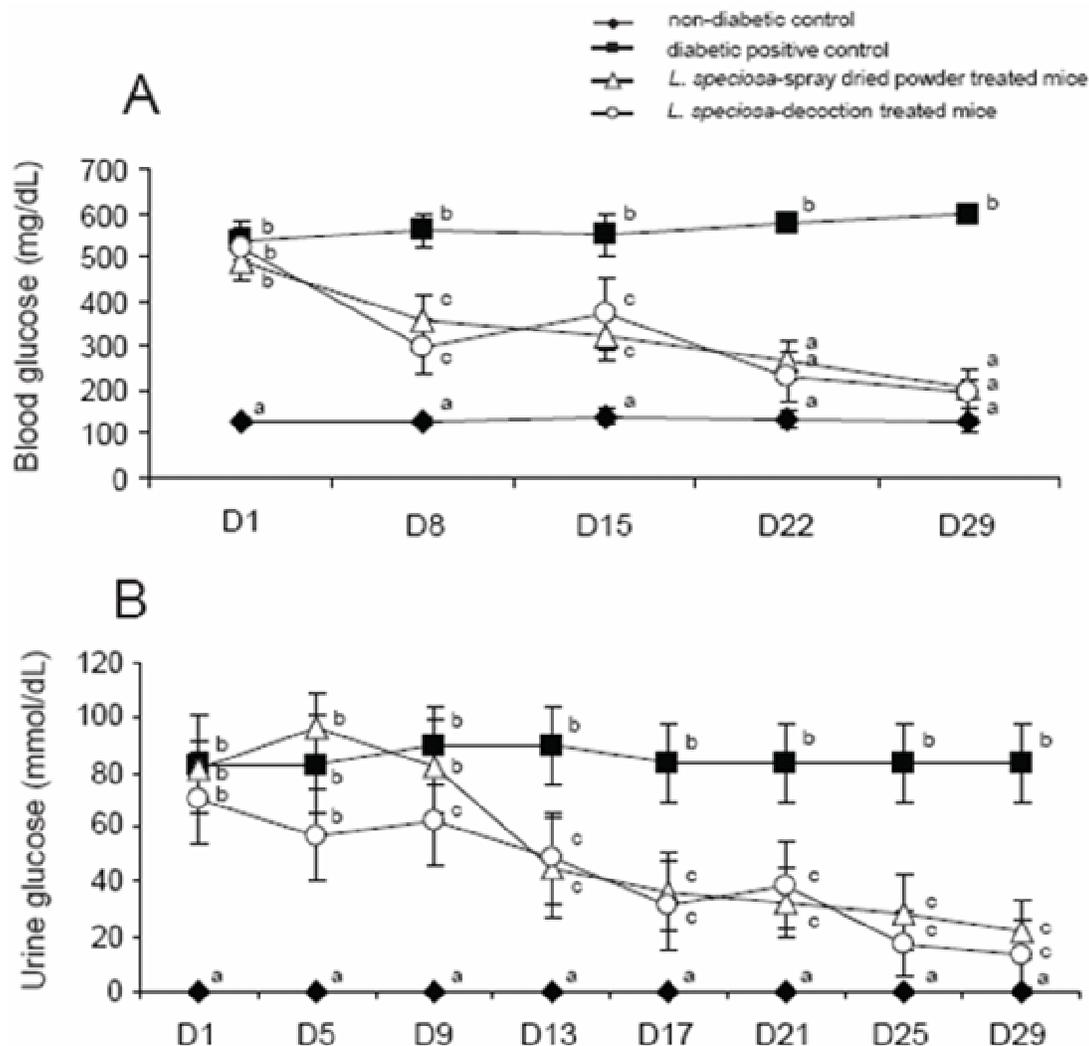
### Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis of the data was performed using a one-way analysis of variance (Graph Pad In Stat, Graph Pad Software Inc., San Diego, CA, USA) followed by post-hoc Tukey-Kramer multiple comparison test.

## RESULTS

Previous studies have shown that *L. speciosa* possesses hypoglycemic activity in alloxan-induced diabetic rats (Mishra, 1990), mice models of diabetes (Kakuda et al., 1996; Suzuki et al., 1999; Takagi et al., 2008) and in diabetic patients (Judy et al., 2003). The present study shows that *L. speciosa* decoction and spray dried powder in alloxan-induced diabetic mice significantly reduced ( $P < 0.01$ ) blood glucose levels on D8 compared with the diabetic control (Figure 1A). Compared with non-diabetic control, the blood glucose levels of alloxan-induced diabetic mice treated with decoction and spray dried powder of *L. speciosa* were higher ( $P < 0.01$ ) at D15, however was comparable with the non-diabetic control from D22 to D28.

Figure 1B presents urinary glucose of alloxan-induced diabetic mice treated with *L. speciosa* decoction and spray dried powder, of the diabetic positive control and non-diabetic control. Glucose was not detected from the urine of non-diabetic control mice throughout the duration of the study. Conversely, glucose was detected in the urine



**Figure 1.** Blood glucose (A) and urine glucose levels (B) of non-diabetic control and alloxan induced-diabetic mice treated and non-treated with *Lagerstroemia speciosa* spray-dried powder and decoction (n = 8 - 11), respectively. Values are mean  $\pm$  SEM. Values with different letters are significantly different from each other in each time point studied (Tukey-Kramer multiple comparisons test after ANOVA).

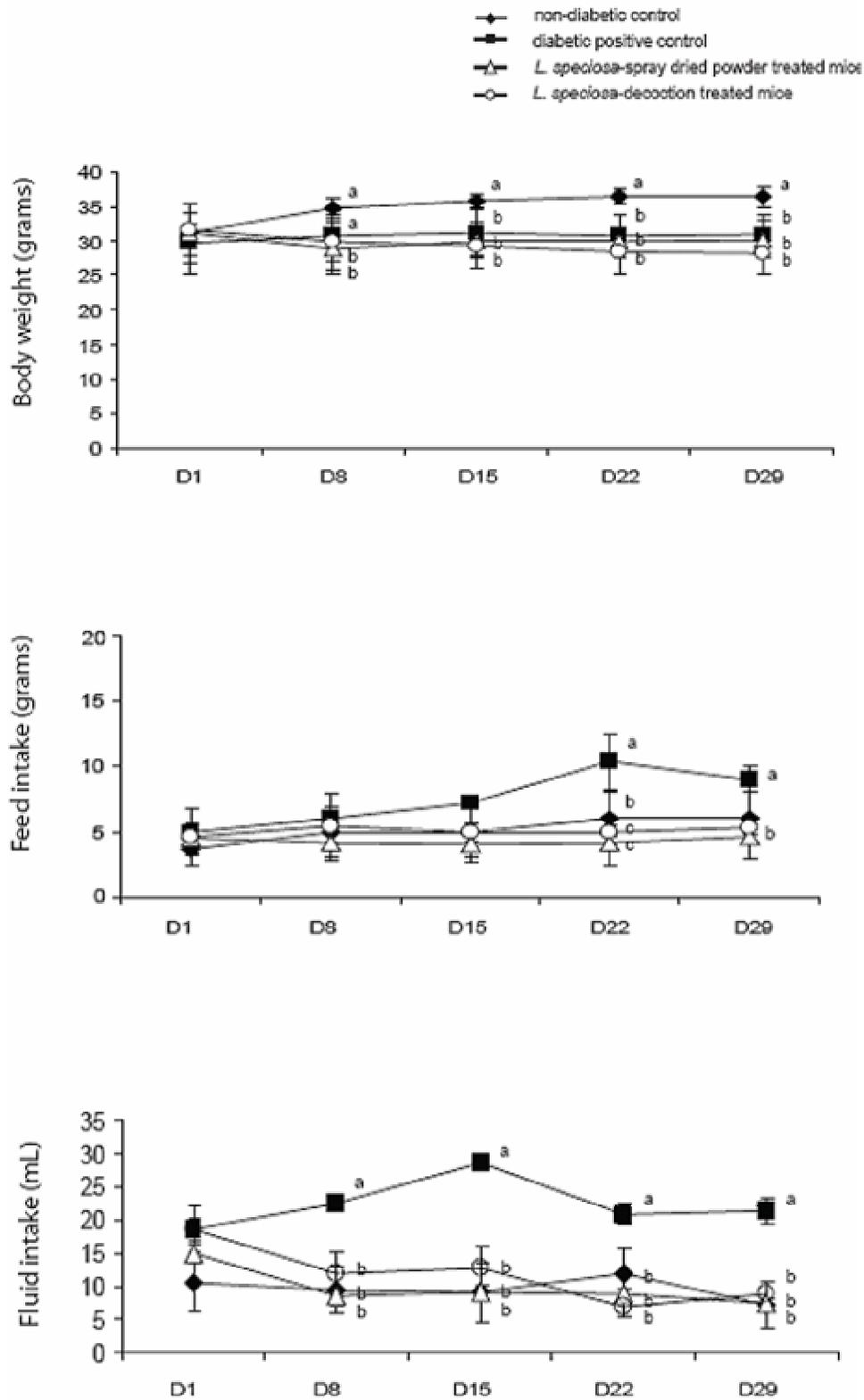
urine of alloxan-induced diabetic mice. Mice treated with *L. speciosa* decoction and spray dried powder had significant reduction ( $P < 0.01$ ) of urinary glucose levels compared with the diabetic positive control from D13 to D29.

Shown in Figure 2 are the body weight, feed intake and water intake of alloxan-induced diabetic mice treated with *L. speciosa* spray dried powder and decoction, of the diabetic positive control and the non-diabetic control. Parameters of growth, feed consumption and water consumption indicated that all groups were comparable at the onset of the experiment. *L. speciosa* spray dried powder and decoction significantly reduced the blood ( $P < 0.01$ ) and urinary glucose ( $P < 0.05$ ) levels in alloxan-induced diabetic mice compared with the diabetic control mice on D8 to D28. These groups of mice had

significantly lower body weight compared with the diabetic control. With regard feed intake, *L. speciosa*-treated mice had lower ( $P < 0.05$ ) consumption compared to diabetic control. A comparable water intake was observed in non-diabetic control and *L. speciosa*-treated mice from D8 to D28 but was significantly lower ( $P < 0.01$ ) compared with the diabetic mice.

## DISCUSSION

As a cytotoxic agent to the insulin-secreting  $\beta$  cells of the pancreas, alloxan effectively induces diabetes in a wide variety of animal models (Reyes et al., 2006; Sun et al., 2008; Xu et al., 2008). Thus, it allows elucidation of antihyperglycemic agents in the treatment of diabetes.



**Figure 2.** Mean body weight (A), feed intake (B) and fluid intake (C) of non-diabetic control and alloxan induced-diabetic mice treated and non-treated with *Lagerstroemia speciosa* spray-dried powder and decoction (n = 8 - 11), respectively. Values are mean  $\pm$  SEM. Values with different letters are significantly different from each other in each time point studied (Tukey-Kramer multiple comparisons test after ANOVA).

Alloxan-induced diabetes consistently produced the main characteristics of diabetes mellitus including polydipsia, polyphagia, polyuria, decreased insulin levels, weight loss and hyperglycemia. This study evaluated the blood glucose levels, urinary glucose, body weight, feed intake and water intake in experimental diabetes induced by alloxan in mice.

In our present study, the significant reduction of blood glucose levels in alloxan-induced diabetic mice treated with *L. speciosa* is in congruence with a previous report demonstrating the anti-hyperglycemic effect of *L. speciosa* in genetically engineered diabetic mouse model (Kakuda et al., 1996). Whereas the genetically diabetic KK-AY mice fed with the control diet had high plasma glucose levels, water consumption, food intake and urinary glucose; the addition of hot water extract from *L. speciosa* leaves in the control diet significantly suppressed the elevation of plasma glucose level in those mice (Kakuda et al., 1996). The blood lowering effect of *L. speciosa* in KK-AY was observed one week following *L. speciosa* administration.

However, in the present study it took at least three weeks to achieve a significant suppression of the elevated plasma glucose level in alloxan-induced diabetic mice from the commencement of *L. speciosa* administration. This disparity could be attributed to the difference in the animal model of diabetes, Kakuda and colleague (Kakuda et al., 1996) used KK-AY, a genetically engineered mouse model while alloxan-induced diabetic mouse model was used in this study. Considering that in studies involving diabetic rat models, the results could be influenced by diabetic induction agents (Sexton and Jarow, 1997), it is likely that a genetically engineered mouse model of diabetes could yield a variation in the results compared to an animal model of chemically induced diabetes. Although both studies used male mice, the age of mice used was different. They used four-week old mice (Kakuda et al., 1996) since the plasma glucose level of KK-AY mice continued to rise gradually until at least 10 weeks old (Odaka et al., 1992) while we used 8 - 10 week old mice.

Furthermore, the kind of preparation and doses used were different. They used *L. speciosa* 5% hot-water extract and 2% methanol eluent fraction while we used the spray dried *L. speciosa* powder at 1000 mg/kg and decoction at 20 ml/kg. A crucial factor that can contribute to the discrepancy could be the role of plant metabolites since these substances tend to considerably change in response to soil, humidity and climate during the growth process (Evans, 2002). Nonetheless, this is the first evidence demonstrating that *L. speciosa* possesses a hypoglycemic potential in an alloxan-induced diabetic mouse model.

Previous studies have shown that biologically active substances obtained from *L. speciosa* including corosolic acid, ellagitannins, lagerstroemin, flosin B and reginin A activate glucose (Hayashi et al., 2002; Murakami et al.,

1993) particularly in rat adipose cells (Hayashi et al., 2002), the physiological target cells of insulin. For example, the tannins efficiently increase the rate of hexose uptake to the level higher than a half of that induced by insulin (Hayashi et al., 2002). Using Chinese hamster ovary cells expressing human insulin receptors, it has been shown that the insulin-like actions of lagerstroemin were accompanied by concomitant increases in tyrosine-phosphorylation of the  $\beta$ -subunits of the insulin receptors (Hattori et al., 2003). Taken together, it is likely that the insulin-like action of the ellagitannins or metabolites could be explained by the binding of tannins with the extracellular domain of the insulin receptors in a fashion causing the activation of the  $\beta$ -subunits of the insulin receptors that in turn could potentially be responsible for the hypoglycemic effect of *L. speciosa* in the present study. However, using alloxan as a diabetic induction agent in mice to define the mechanism by which *L. speciosa* reduces hyperglycemia requires further studies.

In conclusion, the results provide the first physiological evidence that *L. speciosa* possesses anti-hyperglycemic effect in alloxan-induced diabetic mice that may offer a valuable therapeutic measure in the treatment of diabetes mellitus.

## ACKNOWLEDGEMENTS

This project was supported by Southeast Asian Regional Center for Graduate Study and Research in Agriculture scholarship grant to N.C.T. The authors are also thankful to Dr. Zosimo Battad, the former President and Dr. Honorio M. Soriano, Jr., the current President, of Pampanga Agricultural College, Magalang, Pampanga, Philippines for providing support.

## REFERENCES

- Bai N, He K, Roller M, Zheng B, Chen X, Shao Z, Peng T, Zheng Q. (2008). Active compounds from *Lagerstroemia speciosa*, insulin-like glucose uptake-stimulatory/inhibitory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *J. Agric. Food Chem.*, 56:11668-11674.
- de Padua LS, Lugod GC, Pancho JV (1997). Handbook on Philippine Medicinal Plants UPLB Publishing Center, Vol. I. College, Laguna. pp. 64.
- Evans WC (2002). Principles related to the commercial production, quality and standardization of natural products, *In* G. E. Trease and W. C. Evans, eds. *Trease and Evans' Pharmacognosy*, 15th ed. Saunders, London. pp. 55-106
- Fernando ES, Sun BY, Sun MH, Kong HY, Koh KS (2004). Flowering plants and ferns of Mt. Makiling ASEAN-Korean Environmental Unit, Seoul, Korea.
- Hattori K, Sukenobu N, Sasaki T, Takasuga S, Hayashi T, Kasai R, Yamasaki K, Hazeki O (2003). Activation of insulin receptors by lagerstroemin. *J. Pharmacol. Sci.*, 93:69-73.
- Hayashi T, Maruyama H, Kasai R, Hattori K, Takasuga S, Hazeki O, Yamasaki K, Tanaka T (2002). Ellagitannins from *Lagerstroemia speciosa* as activators of glucose transport in fat cells. *Planta Med.*, 68: 173-5.

- Hou W, Li Y, Zhang Q, Wei X, Peng A, Chen L, Wei Y (2009). Triterpene acids isolated from *Lagerstroemia speciosa* leaves as alpha-glucosidase inhibitors. *Phytother. Res.*, 23:614-618.
- Judy WV, Hari SP, Stogsdill WW, Judy JS, Naguib YM, Passwater R (2003). Antidiabetic activity of a standardized extract (Glucosol) from *Lagerstroemia speciosa* leaves in Type II diabetics. A dose-dependence study. *J. Ethnopharmacol.*, 87:115-7.
- Kakuda T, Sakane I, Takihara T, Ozaki Y, Takeuchi H, Kuroyanagi M (1996). Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. leaves in genetically diabetic KK-Ay mice. *Biosci. Biotechnol. Biochem.*, 60:204-8.
- Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. (2007). Antidiabetes and Anti-obesity Activity of *Lagerstroemia speciosa*. *Evid Based Complement Alternat. Med.*, 4:401-407.
- Liu F, Kim J, Li Y, Liu X, Li J, Chen X (2001). An extract of *Lagerstroemia speciosa* L. has insulin-like glucose uptake-stimulatory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *J. Nutr.*, 131:2242-7.
- Mishra YC (1990). Hypoglycemic activity of leaves of *Lagerstroemia speciosa* (L) Pers. *Indian J. Pharmacol.*, 22:174-176.
- Murakami C, Myoga K, Kasai R, Ohtani K, Kurokawa T, Ishibashi S, Dayrit F, Padolina WG, Yamasaki K (1993). Screening of plant constituents for effect on glucose transport activity in Ehrlich ascites tumour cells. *Chem. Pharm. Bull.*, (Tokyo) 41:2129-31.
- Odaka H, Shino A, Ikeda H, Matsuo T (1992). Antiobesity and antidiabetic actions of a new potent disaccharidase inhibitor in genetically obese-diabetic mice, KKA(y). *J. Nutr. Sci. Vitaminol.*, (Tokyo) 38:27-37.
- Quisumbing E (1978). *Medicinal Plants of the Philippines* Katha Publishing, pp. 640-642 Quezon City, Philippines.
- Reyes BAS, Bautista ND, Tanquilut NC, Anunciado RV, Leung AB, Sanchez GC, Magtoto RL, Castronuevo P, Tsukamura H, Maeda KI (2006). Anti-diabetic potentials of *Momordica charantia* and *Andrographis paniculata* and their effects on estrous cyclicity of alloxan-induced diabetic rats. *J. Ethnopharmacol.*, 105:196-200.
- Sexton WJ, Jarow JP (1997). Effect of diabetes mellitus upon male reproductive function. *Urology*, 49:508-515.
- Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF, Xu ZH (2008). Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of *Inonotus obliquus* in submerged culture on normal and alloxan-diabetes mice. *J. Ethnopharmacol.*, 118:7-13.
- Suzuki Y, Unno T, Ushitani M, Hayashi K, Kakuda T (1999). Antiobesity activity of extracts from *Lagerstroemia speciosa* L. leaves on female KK-Ay mice. *J. Nutr. Sci. Vitaminol.*, (Tokyo) 45:791-5.
- Takagi S, Miura T, Ishibashi C, Kawata T, Ishihara E, Gu Y, Ishida T (2008). Effect of corosolic acid on the hydrolysis of disaccharides. *J. Nutr. Sci. Vitaminol.*, (Tokyo) 54:266-8.
- Toledo RT (1980). *Fundamentals of food process engineering* AVI Publishing, Westport, Conn. pp. 409.
- Xu Z, Wang X, Zhou M, Ma L, Deng Y, Zhang H, Zhao A, Zhang Y, Jia W (2008). The antidiabetic activity of total lignan from *Fructus Arctii* against alloxan-induced diabetes in mice and rats. *Phytother. Res.*, 22:97-101.