

Full Length Research Paper

Hypoglycemic activity of *Antidesma bunius* (L.) Spreng and *Mollugo oppositifolia* L. fresh and alcoholic extracts in the db/db diabetic mouse model

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Antidesma bunius (L.) Spreng and *Mollugo oppositifolia* L., also known as “bignay” and “malagoso” respectively, are commonly used medicinal plants in the Philippines to treat diabetes. However, the hypoglycemic effect of *A. bunius* (L.) Spreng and *M. oppositifolia* L. has not been reported to date. Therefore, this study was undertaken to investigate the hypoglycemic effects of the fresh and ethanolic extracts of the plants in db/db diabetic male mice. Fresh and ethanolic extracts (12.5 ml/kg and 250 mg/kg, respectively) were administered by gavage in db/db mouse, an animal model of diabetes. Blood from the tail snip was used to measure blood sugar levels on days 1 (D1), 8 (D8), 15 (D15), 22 (D22) and 29 (D29). Fresh extract of *M. oppositifolia* L. caused a significant reduction of blood glucose levels on D22 compared to untreated db/db mice ($P < 0.05$). On D29, fresh extract of *A. bunius* (L.) Spreng ($P < 0.01$), and fresh ($P < 0.01$) and ethanolic leaf extracts ($P < 0.05$) of *M. oppositifolia* L. caused a significant reduction of blood glucose compared to untreated db/db mice. Fresh and ethanolic extracts of *A. bunius* (L.) Spreng and *M. oppositifolia* L. exhibited a significant hypoglycemic activity in db/db mice.

Key words: *Antidesma bunius* L, Spreng, *Mollugo oppositifolia* L., diabetes, mouse, hypoglycemic.

INTRODUCTION

Diabetes mellitus is the most common chronic endocrine/metabolic disease and considered as one of the 10

leading diseases in the Philippines afflicting one out of every five Filipinos (Philippine diabetes statistics

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(Villasenor and Lamadrid, 2006). A vast majority of Filipinos suffering from diabetes are afflicted with non-insulin dependent diabetes mellitus (Villasenor and Lamadrid, 2006). Characterized by elevated glucose levels that lead to metabolic abnormalities (Sun et al., 2008), diabetes mellitus in 2017 as reported by the International Diabetes Foundation has affected 425 million people globally (IDF Diabetes Atlas, 2017). The increase in prevalence of diabetes globally is projected in 2030 (Sarah et al., 2004), and that includes the Philippines.

Resulting from defects in insulin secretion, insulin action or both (Yki-Jarvinen, 1994), diabetes mellitus continues to pose a significant worldwide burden for the health care system. Associated with long term complications (Searls et al., 2012), diabetes mellitus is classified into two main types. Type 1 results from a deficiency of insulin and is conventionally treated with exogenous insulin, while type 2 results from insulin resistance or reduced insulin sensitivity and is usually treated with oral hypoglycemic agents (Rosak, 2002). Surveys on the use of medicinal plants for treating various diseases indicated a substantial number of respondents utilized herbal medicine (Hajdu and Hohmann, 2012). In the Philippines, despite the dearth of statistics on the efficacy of herbal medicines, complementary and alternative medicine is a traditional practice where diabetes mellitus is commonly treated using medicinal plants (Quisumbing, 1978; Reyes et al., 2017).

Antidesma bunius (L.) Spreng is a shrubby tree belonging to family Euphorbiaceae. Also known as “bignay” in the Philippines (Morton, 1987) and Mao Luang in English (Butkhup and Samappito, 2008). Ripe fruits of *A. bunius* (L.) Spreng contain different types of flavonoids, namely catechin, procyanidin B1 and procyanidin B2 (Butkhup and Samappito, 2008). *Mollugo oppositifolia* L. is a slender, spreading or ascending, smooth branched, annual herb belonging to family Molluginaceae (Quisumbing, 1978). Also known as “malagoso” in the Philippines, *M. oppositifolia* L. contains C-glycosyl-flavonoids (Chopin et al., 1984). Both herbal plants are popular in the Philippines as antidiabetic, however report on phytochemical screening (Doctor and Manuel, 2014) and validation of antihyperglycemic activity (Villasenor and Lamadrid, 2006) of a number of selected indigenous medicinal plants in the Philippines has not included *A. bunius* (L.) Spreng and *M. oppositifolia* L. In addition, *A. bunius* (L.) Spreng and *M. oppositifolia* L are not included in the list of approved herbal medicine by the Department of Health in the Philippines.

Previous studies have shown that *A. bunius* (L.) Spreng has been used in traditional medicine for the treatment of various conditions (Udomkasemsab et al., 2018).

Although the hypoglycemic effects of *A. bunius* (L.) Spreng has been investigated in alloxan- (El-Tantawy et

al., 2015) and streptozotocin- (Chowtivannakul et al., 2016) induced diabetic rat model, it has not been elucidated in db/db diabetic male mice. Some species of Molluginaceae has been demonstrated to exhibit a variety of activities including anti-inflammatory (Kim et al., 2008), antibacterial (Kim et al., 2008), spermicidal (Padma and Khosa, 1995), antifungal (Rajasekaran et al., 1993), immunomodulatory (Ferreira et al., 2003) and antioxidant (Lin et al., 2004). However, to our knowledge, the anti-diabetic potential of *M. oppositifolia* L. has not been elucidated. In the present study, the anti-diabetic potential of *A. bunius* (L.) Spreng and *M. oppositifolia* L. was investigated in an animal model of diabetes, the db/db mouse.

MATERIALS AND METHODS

Plant

Fruits and leaves of *A. bunius* (L.) Spreng and *M. oppositifolia* L. were collected in Pampanga State Agricultural University, Magalang, Pampanga, Philippines. Plant materials were identified and authenticated at the Botanical Herbarium, Museum of Natural History, University of the Philippines, Los Baños, College, Laguna, Philippines by Annalee S. Hadsall.

Animals

Three-month old db/db diabetic male mice weighing 37 to 44 g (n = 5/group) were used in this study. They were caged individually on a 12-h light schedule in a temperature controlled (20±2°C) colony room. They were given food and water *ad libitum*. The mice were treated in accordance with the guidelines set by the Institutional Animal Care and Use Committee of Pampanga Agricultural College. All efforts were made to utilize only the minimum number of animals necessary to produce reliable scientific data.

Preparation of fruit and leaf extracts

Fresh fruits of *A. bunius* (L.) Spreng and fresh leaves of *M. oppositifolia* L. were carefully washed, cut and dried at 50°C. The fruits and leaves were powdered (100 g) and mixed with 500 mL of 80% ethanol in round bottomed Erlenmeyer flask, soaked for 48 h and filtered. Using a rotary evaporator (Heidolph^R Rotary Evaporator, Schwabach, Germany) filtrated extracts were concentrated and the ethanol was removed. The percent yield of extract was calculated by dividing the weight of the extract by the weight of plant material multiply by 100.

Phytochemical screening for flavonoids

One hundred gram of air-dried fruits of *A. bunius* (L.) Spreng and leaves of *M. oppositifolia* L. were ground to fine powder, soaked in 80% ethanol for 48 h, filtered and concentrated *in vacuo*. The presence of flavonoids was determined by conducting the Bate-Smith and Metcalf method and Wilstatter “cyanidin” test as previously described (Guevarra, 2005) and utilized (Doctor and Manuel, 2014). Briefly, Bate-Smith and Metcalf method was carried out with the treatment of the ethanolic extract with hydrochloric acid (HCl) while the Wilstatter “cyanidin” test was carried out by using 1% aluminium chloride solution in methanol concentrated HCl, magnesium turnings and potassium hydroxide solution (Guevarra,

2005).

Acute toxicity test

Eighty mice of both sexes were grouped into four with five mice per group. The fresh extracts were prepared by obtaining 250 g fresh fruits of *A. buni* (L.) Spreng and 250 g fresh leaves of *M. oppositifolia* L. The fresh leaves were osterized and filtered with a clean cheese cloth. The fresh extracts were placed in clean bottles and were administered orally using a gavage at the dose of 6.25, 12.5, 18.75 and 25 ml/kg. The ethanolic extracts were given at 100, 250, 500 and 1000 mg/kg. Mice were given feed and water *ad libitum*. Signs of toxicity and percentage mortality were observed over 48 h.

Treatments

The db/db diabetic male mice were randomly divided into five groups consisting of five mice each. The mice in the first group were given distilled water and served as the diabetic control. The second and third groups were given fresh and ethanolic fruit extracts of *A. buni* (L.) Spreng at a concentration of 12.5 ml/kg and 250 mg/kg, respectively. The fourth and fifth groups were given fresh and ethanolic leaf extracts of *M. oppositifolia* L. at a concentration of 12.5 ml/kg and 250 mg/kg, respectively. Fruit and ethanolic extracts were administered via gavage daily (at 1700 h).

Data collection

The blood glucose levels were measured on day 1 (D1), day 8 (D8), day 15 (D15), day 22 (D22) and day 29 (D29). Blood samples were collected from the tail vein and measured with a glucometer (OneTouch^R Ultra^R, LifeScan, Inc., Milpitas, CA, USA). The reduction in blood glucose levels was calculated by subtracting the blood glucose level of mouse on D22 or D29 from D1, and dividing it to the glucose level on D1 multiply by 100.

Statistical analysis

All data were expressed as mean $\pm$ standard error of mean (SEM). The effect of *A. buni* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts on blood glucose levels was determined using one way-analysis of variance (Graph Pad In Stat, Graph Pad Software Inc., San Diego, CA, USA) followed by post-hoc Tukey-Kramer multiple comparisons test.

RESULTS

Percentage yield and phytochemical screening of *A. buni* (L.) Spreng and *M. oppositifolia* L.

The yield of the ethanolic fruit extract of *A. buni* (L.) Spreng was 13.52% (w/w) dry matter and was reddish purple in color while the ethanolic leaf extract of *M. oppositifolia* L. was 11.73% (w/w) dry matter and was dark green in color. Using the Bate-Smith and Metcalf method, and Wilstatter "cyanidin" test (Guevarra, 2005; Doctor and Manuel, 2014) revealed that flavonoids were detected in ethanolic extracts of *A. buni* (L.) Spreng and *M. oppositifolia* L.

Acute toxicity test

The acute toxicity test of fruit and leaf extracts of *A. buni* (L.) Spreng and *M. oppositifolia* L. in mice did not produce mortality or signs of toxicity even with the highest dose of extract (1000 mg/kg) administered orally throughout the observation period of 48 h. The absence of mortality or signs of toxicity indicates that the fresh and ethanolic fruit or leaf extract as well as the dose used in the present study were safe in animals.

Hypoglycemic effect of *A. buni* L and *M. oppositifolia* L

Data on the blood glucose levels of db/db mice following treatment with *A. buni* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts are presented in Figure 1.

The results showed that from D1 to D15, no significant differences were observed between groups on the blood glucose levels in db/db mice treated with *A. buni* (L.) Spreng and *M. oppositifolia* L. fresh and ethanolic extracts. However, on D22 a significant reduction of blood glucose levels in db/db mice treated with *M. oppositifolia* L. fresh extract was evident compared with the untreated db/db diabetic mice ($P < 0.05$). On D29, a significant reduction in blood sugar levels was observed in db/db mice treated with *A. buni* (L.) Spreng fresh extract ($P < 0.01$) and *M. oppositifolia* L. fresh ($P < 0.01$) and ethanolic extracts ($P < 0.05$) compared with untreated db/db diabetic mice. These decreased the blood glucose levels by 79% with *A. buni* (L.) Spreng fresh extract, 79 and 55%, with *M. oppositifolia* L. fresh and ethanolic extracts, respectively (Table 1).

DISCUSSION

The hypoglycemic activity of *A. buni* (L.) Spreng and *M. oppositifolia* L. was evaluated in db/db mouse model of diabetes. The present results confirm previous investigations demonstrating that *A. buni* (L.) Spreng exhibits antidiabetic activity in streptozotocin- (Chowtivanakul et al., 2016) and in alloxan- (El-Tantawy et al., 2015) induced diabetic rats and further extend the findings on the antidiabetic activity of *A. buni* (L.) Spreng in a mouse model of diabetes, db/db mice. Importantly, the present results show that *M. oppositifolia* L. exhibits antidiabetic activity in db/db mice. To our knowledge, this is the first *in vivo* evidence showing the antidiabetic activity of *M. oppositifolia* L. in db/db mice.

Consistent with previous reports, hyperglycemia is evident in db/db mice (Huynh et al., 2012). In the present study, untreated db/db mice all exhibited marked hyperglycemia throughout the duration of the study. Specifically on D29, untreated db/db mice had significantly higher blood glucose levels compared with db/db mice treated with *A. buni* (L.) Spreng fresh

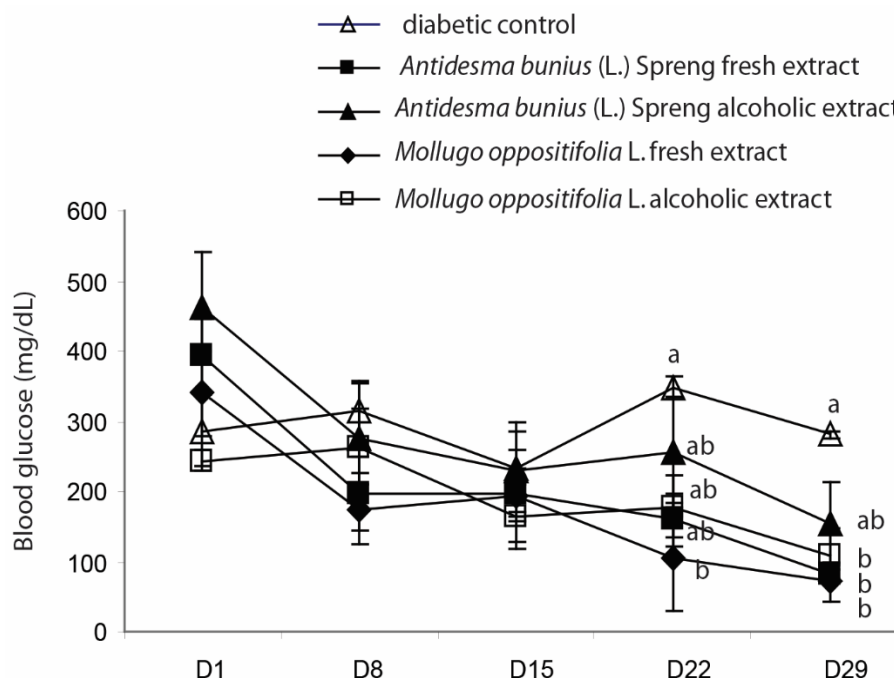


Figure 1. Blood glucose of untreated db/db diabetic control and db/db diabetic mice treated with fresh and ethanolic extracts of *Antidesma bunius* (L.) Spreng and *Mollugo oppositifolia* L. (n=5). Values are means $\pm$ SEM. Values with different letters are significantly different from each other in each time point studied (Tukey–Kramer multiple comparisons test after ANOVA). D1, Day 1; D8, Day 8; D15, Day 15; D22, Day 22; D29, Day 29.

Table 1. Percentage reduction of blood glucose levels following *Antidesma bunius* L and *Mollugo oppositifolia* L treatment in db/db mice.

Treatment	Blood glucose (mg/dL)			Percentage reduction	
	Day 1	Day 22	Day 29	Day 22	Day 29
Diabetic control	286.2	348.6	280.8	-	-
<i>Antidesma bunius</i> fresh extract	393	159.2	82.4	59.5	79.0
<i>Antidesma bunius</i> alcoholic extract	462.2	256.2	154.8	44.6	66.5
<i>Mollugo oppositifolia</i> fresh extract	339.4	106.2	71	66.4	79.1
<i>Mollugo oppositifolia</i> alcoholic extract	243.4	178.4	109.6	26.7	55.0

extract and *M. oppositifolia* L. fresh and ethanolic extracts. The potential mechanism by which *A. bunius* (L.) Spreng and *M. oppositifolia* L. reduced the blood glucose level is not clearly defined. Given that the present study was limited to investigating whether *A. bunius* (L.) Spreng and *M. oppositifolia* L. demonstrate an anti-diabetic potential in a mouse model of diabetes, the specific mechanisms by which *A. bunius* (L.) Spreng and *M. oppositifolia* L. decrease the elevated blood glucose levels could not be conclusively described. However, based on previous studies, we proposed that *A. bunius* (L.) Spreng and *M. oppositifolia* L. could lower blood glucose levels by increasing insulin input or inhibiting the

intestinal absorption of glucose (Marviya et al., 2010). These potential mechanisms could lead to the restoration of the pancreatic tissue function (Marviya et al., 2010), and consequently lowers the elevated blood glucose level. Moreover, it is likely that *A. bunius* (L.) Spreng and *M. oppositifolia* L. may have attenuated death of β cells in the db/db mouse since the use of medicinal plant in this strain was associated with significant increases in insulin content accompanied with preservation of β cell architecture (Huynh et al., 2012). Therefore, protection of functional β cell would also mean preservation of insulin production, thereby decreasing the elevated blood glucose level.

Medicinal plants containing active biological principles including flavonoids, and tannins have been reported to demonstrate hypoglycemic properties (Suba et al., 2004). In particular, the therapeutic potential of flavonoids displays significant insulin secretagogue, insulinomimetic and cytoprotective effects (Bharucha et al., 2011). Considering that *A. bunioides* (L.) Spreng contains flavonoids (Butkhup and Samappito, 2008) and *M. oppositifolia* L. contains C-glycosyl-flavonoids (Chopin et al., 1984), it is tempting to speculate that the active biological principles present in the plant may be responsible for the hypoglycemic effects observed in the present study.

Conclusion

Overall, the present study provides the first physiological evidence that *A. bunioides* (L.) Spreng and *M. oppositifolia* L. possess significant antidiabetic activity in db/db diabetic mouse model. The results may offer a valuable therapeutic potential in the treatment of diabetes mellitus; however, further investigations are required to address the exact mechanism by which *A. bunioides* (L.) Spreng and *M. oppositifolia* L. lower elevated blood glucose levels in diabetic mice.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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