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Research Article

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Hypoglycemic activity of ethanolic and aqueous extracts of *Antides mabunius* fruits on alloxan-induced hyperglycemic ICR mice

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ABSTRACT

Diabetes mellitus is a chronic health condition in which the body either fails to produce sufficient amount of insulin or it responds abnormally to insulin. Current treatments, besides proper diet and exercise, include sulfonylureas, meglitinide, biguanide, thiazolidinedione, and alpha-glucosidase inhibitors; however, these are costly and not without side effects. The present study explores the hypoglycemic properties of Antides mabunius fruit ethanolic and aqueous extracts on alloxan-induced hyperglycemic ICR mice. For this study, diabetic mice were treated with 100 mg/kg, 300 mg/kg or 500 mg/kg aqueous fruit extract or ethanolic fruit extract via oral gavage and observed for 14 days. Measurement of their Fasting Blood Glucose levels (FBG) on the 3rd, 7th, 10th and 14th days show that all treatments were able to lower FBG levels, with 500 mg/kg ethanolic fruit extract being the most effective. Preliminary phytochemical screening shows positive results for phenols, indoles, steroids and flavonoids. The study shows that of A. bunius ethanolic fruit extract as a potential herbal drug candidate for the treatment of diabetes.

Keywords: *Antides mabunius* fruits, ethanolic and aqueous extracts, hypoglycemic effect, ICR mice, alloxan-induced hyperglycemia

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases wherein the body fails to produce enough insulin or that the cells themselves respond abnormally to insulin; thus, heightened blood glucose levels are observed in individuals with diabetes[1]. Besides hyperglycemia, increased frequency or severity of infections have also been associated with diabetes, like mucormycosis, cystitis, urinary tract infections, intrarenal abscesses, pneumonia, emphysematous cholecystitis and malignant otitis externa [2]. Aside from these, diabetes has also been associated with other body malfunctions life in the eyes, kidneys, nerves, heart and blood vessels [3].

According to Shaw et al, the world prevalence of diabetes for the year 2010 was 6.4% among adults, affecting 285 million [4]. They projected that in 2030, the number would increase to 7.7%, affecting 439 million by then.

Current treatment methods include the use of synthetic drugs, like sulfonylureas, meglitinide, biguanide, thiazolidinedione and alpha-glucosidase inhibitors. However, using these drugs is costly and not without side-effects such as nausea, vomiting, diarrhea, dizziness, headache, acidity, hypersensitivity, abdominal upset, weakness, sinusitis and muscle pain [5]. Thus, for the past decades scientists have been exploring plant products as potential diabetes treatments. Currently, an estimated 1200 plants have been sown to possess some antidiabetic properties [6]. Several of these can be found in the Philippines: *Momordica charantia* and *Andrographis paniculata*[7].

Antides mabunius, or*bignay (Filipino local name)*, is an ubiquitous invasive shrub tree with wide spreading branches, alternate leaves, and round fruits borne in grape-like pendent clusters. Although the bark is considered poisonous, the people in Thailand use *A. bunius* as medicinal plant for gastric intestinal problems, dysentery, indigestion, and constipation. Compounds in *A. bunius* plants have been observed to possess antimicrobial [8] and antioxidant [9] activities.

The present study aims to demonstrate the hypoglycemic activity of *A. bunius* fruit ethanolic and aqueous extracts on alloxan-induced hyperglycemic International Cancer Research (ICR) Strain mice. The study also aims to determine groups of plant metabolites that may be responsible for the hypoglycemic activity of the plant.

EXPERIMENTAL SCETION

Plant sample procurement and preparation

Three- to four-week old *A. bunius* fruits were obtained from Rosario, Cavite, Philippines and identified by Bureau of Plant Industry, Quirino Ave., Manila, Philippines. The fruits were washed with water and then air-dried. Dried fruits were powdered and stored under room temperature until further use.

Animal sample procurement

Nine-week old ICR strain mice weighing 25-30 grams were obtained from the Food and Drug Administration (FDA), Alabang, Muntinlupa City, Philippines. The mice were caged individually and given rabbit pellets and distilled water *ad libitum* as their diet. Prior to the assay, the mice were acclimatized for one week.

Extraction of *A. bunius* fruits

Five hundred gram portions of the powdered fruits were immersed separately into 2 L distilled water and 80% ethanol for 24 hours, with occasional stirring. After which, the solutions were filtered, collecting the filtrates. The filtrates were lyophilized and the residues obtained were stored in 4℃ until further use. Test solutions used for treating the mice were prepared by dissolving the residues in distilled water.

Acute Toxicity Test

Prior to hypoglycemic activity assay, the extracts were tested for acute toxicity. Six female ICR mice were randomly selected for this assay. The mice were given 2000 mg extract/kg body weight by oral gavage and then observed for mortality after 14 days.

Induction of Diabetes

Prior to induction, 45 ICR mice were fasted for 4-5 hours and had their basal Fasting Blood Glucose (FBG) levels determined using OneTouch Ultra Glucometer^{TM}(Johnson & Johnson Co., USA). Then, 2% alloxan in Normal Saline Solution (0.9% NaCl) were injected intraperitoneally to the mice at 2 mL/100 g body weight. Afterwards, the mice were given 5% glucose stock solution overnight to counter hypoglycemic shock. Two days after alloxan injection, the FBG levels were again determined. Since all mice had FBG levels greater than 200 mg/dL, indicating hyperglycemia, all of these were used for further studies. The mice were allowed to stabilize for 3 more days before further experimentation.

Table 1. Treatment groups used in the study

Group Number*	Label	Treatment
1	Normal	Normal, Treated with distilled water
2	Metformin	Hyperglycemic, Treated with positive control (Metformin)
3	DH_2O	Hyperglycemic, Treated with negative control (DH_2O)
4	A1-100	Hyperglycemic, Treated with 100 mg Aqueous extract/kg body weight
5	Aq-300	Hyperglycemic, Treated with 300 mg Aqueous extract/kg body weight
6	Aq-500	Hyperglycemic, Treated with 500 mg Aqueous extract/kg body weight
7	EtOH-100	Hyperglycemic, Treated with 100 mg Ethanolic extract/kg body weight
8	EtOH-300	Hyperglycemic, Treated with 300 mg Ethanolic extract/kg body weight
9	EtOH-500	Hyperglycemic, Treated with 500 mg Ethanolic extract/kg body weight

** Each group contains 5-6 female 9-week old ICR strain mice. Prior to assay, the mice were acclimatized for a week.*

Treatment Allocation and FBG level determination

The 45 hyperglycemic mice and 5 normal mice were divided into different treatment groups as shown in **Table 1**. Prior to treatment, the mice were fasted for 4-5 hours. The treatments were administered via oral gavage starting three days after induction of diabetes. FBG levels were determined on the 3rd, 7th, 10th and 14th day after administering the treatments. These were determined via tail vein puncture and OneTouch Ultra

Glucometer™(Johnson & Johnson Co., USA). The weight of each mouse was also determined on the 3rd, 7th, 10th and 14th day after treatment.

Phytochemical Screening

The aqueous and ethanolic fruit extracts were tested for the presence of alkaloids, steroids, anthraquinones, tannins, phenols, indoles, flavonoids and cardenolides using spray tests. These were spotted on TLC plates and developed under either chloroform: acetic acid: water (50:45:5) or butanol: acetic acid: water (4:1:5) until chromatograms developed. The plates were sprayed with the appropriate spray reagent and color changes were noted.

Statistical Tests

Results were analyzed using pair-wise comparisons (independent sample *t-tests*). These were carried using Statistical Package for the Social Sciences (SPSS) ver. 17.

RESULTS AND DISCUSSION

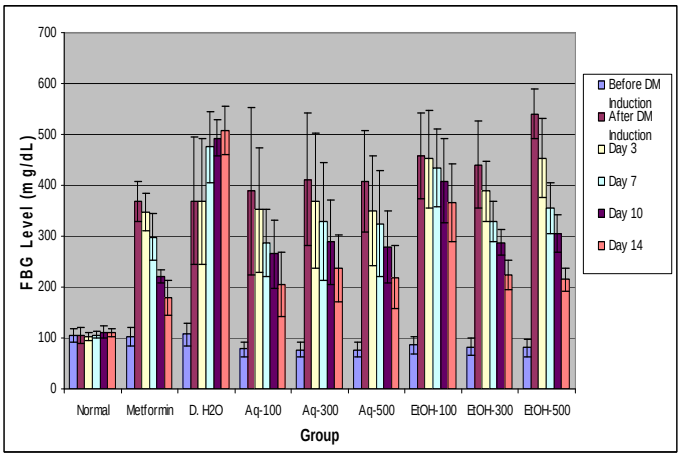

Figure 1. Histogram of the FBG levels of alloxan-induced diabetic mice in each group before and after alloxan injection, and 3rd, 7th, 10th, and 14th day after administration of *A. bunius*extracts and metformin

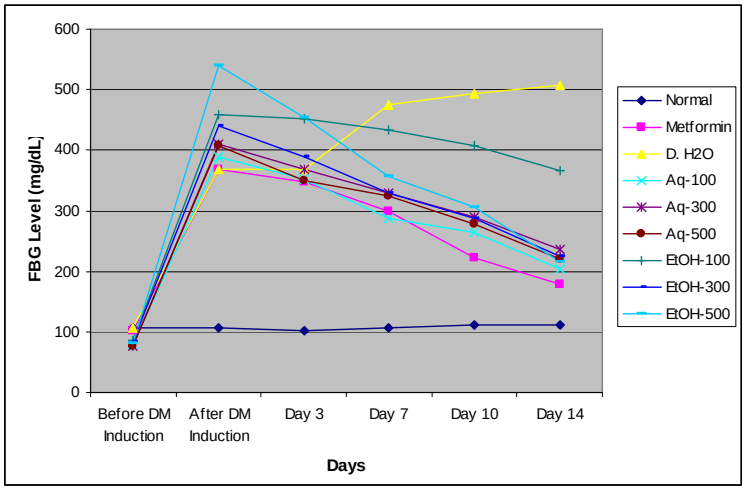

Figure 2 Line graph of the FBG levels of alloxan-induced diabetic mice in each group before and after alloxan injection, and 3rd, 7th, 10th, and 14th day after administration of *A. bunius* extracts and metformin

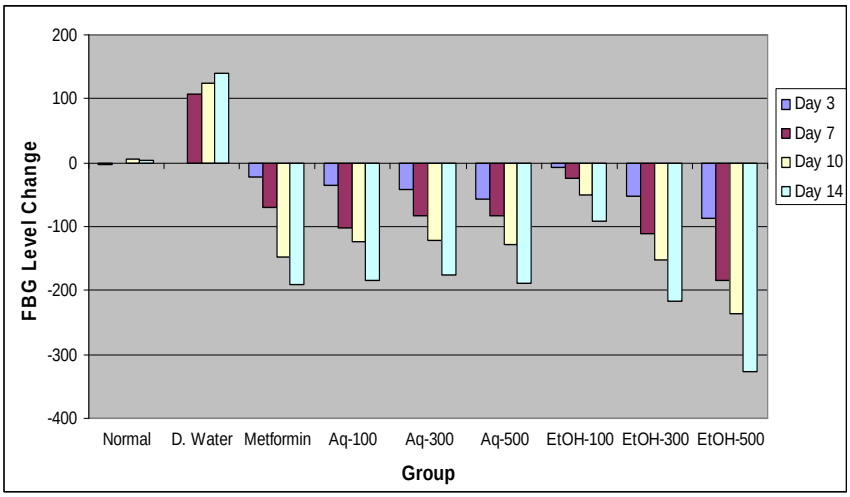

Figure 3. Histogram of average change in FBG values of mice 3rd, 7th, 10th, and 14th days after treatment of the aqueous and ethanolic extracts and metformin

Table 2. FBG levels of alloxan-induced diabetic mice in each group before and after alloxan injection, and after administration of *A. bunius* extracts and metformin.

Group	FBG Level (mg/dl)					
	Before alloxan injection	After alloxan injection	Days After Treatment			
			Day 3	Day 7	Day 10	Day 14
Normal	105.6 ± 13.78	106.0 ± 15.70	102.4 ± 8.69	105.6 ± 6.56	111.2 ± 12.34	110.2 ± 8.70
Metformin	102.2 ± 18.53	369.0 ± 38.86	347.4 ± 37.05	298.4 ± 46.14	222.0 ± 12.88	178.4 ± 33.55
D. H2O	106.6 ± 22.22	368.6 ± 124.97	368.6 ± 123.37	475.6 ± 69.07	492.8 ± 35.90	508.4 ± 47.75
Aq-100	78.0 ± 15.23	388.2 ± 165.36	352.4 ± 122.44	286.8 ± 66.13	264.6 ± 68.27	204.4 ± 63.59
Aq-300	77.4 ± 14.15	411.0 ± 129.84	369.4 ± 133.44	328.6 ± 115.97	288.8 ± 82.65	235.8 ± 65.79
Aq-500	77.6 ± 15.53	407.4 ± 99.95	349.8 ± 108.58	324.0 ± 103.68	278.6 ± 71.66	219.4 ± 62.70
EtOH-100	86.4 ± 16.75	458.6 ± 84.69	451.6 ± 95.17	434.2 ± 77.50	408.4 ± 83.26	366.4 ± 76.72
EtOH-300	82.4 ± 17.51	440.6 ± 84.99	388.6 ± 59.36	328.8 ± 40.62	288.0 ± 24.29	223.8 ± 28.11
EtOH-500	80.6 ± 17.74	540.4 ± 48.52	453.8 ± 78.55	355.8 ± 49.76	305.2 ± 36.78	214.6 ± 23.54

Table 3. Mean body weights of alloxan-induced diabetic mice in each group before and after alloxan injection, and 3rd, 7th, 10th, and 14th day after administration of *A. bunius* extracts and metformin

Group	Body Weight (g)					
	Before alloxan injection	After alloxan injection	Days After Treatment			
			Day 3	Day 7	Day 10	Day 14
Normal	24.04	22.22	23.02	24.46	24.64	23.90
Metformin	26.56	25.16	25.98	24.62	27.60	23.78
D. H2O	25.34	24.92	24.78	23.34	23.04	21.60
Aq-100	22.76	20.40	21.12	20.18	20.88	21.20
Aq-300	27.14	25.56	27.90	26.16	25.72	26.48
Aq-500	26.06	22.78	22.56	22.38	20.28	19.98
EtOH-100	25.60	24.12	25.46	23.32	22.56	21.06
EtOH-300	25.78	25.28	25.24	24.96	25.04	25.58
EtOH-500	24.48	22.60	22.38	22.32	22.04	20.96

Table 4. The bioactive components of aqueous and ethanolic *A. bunius* extracts based on the phytochemical screening performed

Constituent Tested	Test Reagent	Result*	
		Ethanolic extract	Aqueous Extract
Steroids	Vanillin-sulfuric acid	+	+
Anthraquinones	Magnesium acetate in methanol	+	+
Tannins	Potassium-ferricyanide-ferric chloride	+	+
Phenols	Potassium-ferricyanide-ferric chloride	+	+
Indoles	Van-Urk-Salkowski Test	+	+
Flavonoids	Potassium-ferricyanide-ferric chloride	+	+
Alkaloids	Dragendorff's Reagent	-	-
Cardenolides	Kedde Reagent	-	-

'+': present, '-': absent

After lyophilization, 6.62 g aqueous extracts and 7.78 g ethanolic extracts were obtained. These give 1.324% and 1.556% yields for the aqueous and ethanolic extracts respectively. Also, the mice treated with 2000 mg extracts/kg

body weight had zero mortalities and no abnormal behaviors for the duration of the experiment (14 days), implying that the extract is safe to be administered to the mice.

Table 2 shows the average FBG levels for each group (expressed as Mean ± Std. dev.) while **Figures 1 and 2** show the histograms and line graphs of the FBG levels, respectively. All mice treated with alloxan showed increased in their FBG levels about three times as compared to their baseline FBG levels. This signified that the induction of diabetes was successful. As expected, the normal untreated mice did not have any significant changes in their FBG levels. Treatment of the extracts as well as metformin significantly decreased the FBG levels of mice during the 3rd, 7th, 10th, and 14th day following the administration. The diabetic untreated mice, as anticipated continued to display increase in their FBG levels during the same period of time. **Figure 3** shows the average change in FBG level on the 3^{rd}, 7^{th}, 10^{th} and 14^{th} day after treatment. Analysis of the data showed two trends in the FBG levels of mice: (1) FBG levels decreased in metformin and all aqueous and ethanolic extract-treated groups as the day passed by; (2) the decrease in FBG level became higher as the dose increased for ethanolic extract-treated mice. The second trend however was not observed in the case of aqueous extract.

The FBG level data was analyzed using SPSS statistical software with the FBG levels on the 3rd, 7th, 10th, and 14th day after administration of the extracts as the dependent variables. FBG levels before and after alloxan injection were considered independent variables since these data were unaffected by the administration of the extracts together with metformin on the hyperglycemic mice. High dose (500mg/kg) of the ethanolic extract was found to cause the largest mean decrease in FBG of diabetic mice. Pairwise comparison of this group with the other groups revealed that the differences are all significant at 95% confidence interval, implying that 500 mg/kg dosage of ethanolic extract had the most significant effect in lowering the FBG levels of mice ($p\text{-value}<0.05$ when compared against the other treatments). Additionally, the hypoglycemic activity seems to be dose-dependent in the ethanolic extracts, since there are significant differences between the 500 mg/kg, 300 mg/kg and 100 mg/kg differences ($p\text{-value}<0.05$), with 500 mg/kg being the most active, followed by 300 mg/kg and lastly 100 mg/kg. On the other hand, the aqueous extracts also have significant hypoglycemic activity compared to the negative and positive controls, but the effect is not dose-dependent ($p\text{-value}>0.05$).

Mean body weights of the animals were also determined after 3^{rd}, 4^{th}, 7^{th}, 10^{th} and 14^{th} day of treatment. **Table 3** shows the average body weights of the mice for the duration of the experiment. However, further statistical analysis shows no relationship between FBG levels and body weight.

Preliminary phytochemical screening showed the presence of steroids, anthraquinones, tannins, phenols, indoles and flavonoids (**Table 4**) for both extracts. Some studies however discovered that *A. bunius*fruits contain flavonoid compounds. The major flavonoids in *A. bunius* fruits include catechin, procyanidin B1, and procyanidin B2 [10]. In another study conducted, it was found out that fractions from plants containing steroid and coumarin were the ones with the anti-hyperglycemic property on normal and alloxan-induced diabetic mice as observed from serum glucose level and liver glycogen content [11]. Another study suggest that alkaloids, flavonoids, cardiac glycosides, and saponins are also anti-diabetic agents [12] while tannins act as alpha-glucosidase inhibitors which means that they reduce the absorption of carbohydrates in the gut. In addition to this, tannins and polyphenolics were discovered to be potent antioxidants and have strong scavenging activity against ROS [13].

Peroxisome proliferators-activated receptor gamma (PPAR-gamma) is a receptor protein that regulates the uptake of glucose in fat cells. A drug called rosiglitazone is an anti-diabetic drug that targets PPAR-gamma in fat. When rosiglitazone binds to the receptor, it makes them more sensitive to insulin and improves the uptake of glucose. Some flavonoids like the epicatechingallate, and polyphenols like ellagic acid, were found to have high affinity to PPAR-gamma. When these compounds bind to the receptor, they act like rosiglitazone and activate PPAR-gamma resulting to greater glucose uptake [14]. The presence of these components therefore might have been the reason for the observed lowering of the FBG levels in diabetic mice after treatment of the extract. Further studies must be conducted to verify this claim.

CONCLUSION

In conclusion, the hypoglycemic effect of *A. bunius* ethanolic and aqueous fruit extracts have been demonstrated on alloxan-induced hyperglycemic ICR mice. Aqueous and ethanolic extracts at 100, 300 and 500 mg extract/kg body weight were found to significantly lower FBG levels on the 3^{rd}, 7^{th}, 10^{th} and 14^{th} day after introduction of treatments. Furthermore, increasing the dosage of ethanolic treatment significantly decreased the FBG. The highest average decrease in FBG level was observed when the diabetic mice were treated with 500 mg/kg body weight of the ethanolic extract. This dose of the extract was found to be more effective than metformin. Phytochemical analysis revealed the presence of steroids, anthraquinones, tanins, flavonoids, phenols and indoles. These constituents,

particularly the flavonoids, steroids, phenols and tannins may have been responsible for the extracts' activities. Thus, the study showed *A. bunius* as potential source of hypoglycemic compounds. The group is now working on the isolation, purification, and identification of the compounds responsible for the hypoglycemic property of *A. bunius* extract.

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REFERENCES

[1] American Diabetes Association,*Diabetes Care*, **2004**, 27(Suppl. 1), S5-S10.
[2] D Schaberg; M Norwood,*Diabetes Spectr.*,**2002**, 15(1), 37-40
[3] K Kadhirvel; P Rajivgandhi; G Narayanan; V Govindaraji; K Kannan; R Vanithaselvi; S Ramya; R Jayakumararaj,*Ethnobot. Leaflets*, **2010**, 14, 236-247.
[4] J Shaw; R Sicree; P Zimmet,*Diabetes Res. Clin. Pract.*,**2010**, 87(1), 4-14.
[5] S Singh; S Gupta; G Sabir; M Gupta; P Seth,*Bioinformation*, **2009**, 4(6), 263-268.
[6] N kambouche; B Merah; A Derdour; S Bellahouel; J Bouayed; A Dicko; C Younos; R Soulimani,*Afr. J. Biotechnol.*, **2009**, 8(20), 5589-5594.
[7] B Reyes; N Bautista; N Tanquilut; R Anunciado; A Leung; G Sanchez; R Magtoto; P Castronuevo; H Tsukamura; K Maeda,*J. Ethnopharmacol.*, **2006**, 105(1-2), 196-200.
[8] R Lizardo; L Mabesa; E Dizon; N Aquino,*Int. Food Res. J.*, **2015**, 22(1), 88-95.
[9] SJorjong; L Butkhp; S Samappito,*Food Chem.*, **2015**, 181, 248-255.
[10] L Butkhup; S Samappito,*Pak. J. Biol. Sci.*, **2008**, 11(7), 996-1002.
[11] N Ajikumaran; B Shylesh; B Gopakumar; ASubramoniam,*J. Ethnopharmacol.*, **2006**, 106(2), 192-197.
[12] G Adebayo; O Alabi; B Owoyele; A Soladoye,*Rec. Nat. Prod.*, **2009**, 3(4), 187-192.
[13] O Said; S fulder; K Khalil; H Azaizeh; E Kassis; B Saad,*Evid. Based Complement. Alternat. Med.*, **2008**, 5(4), 421-428.
[14] A Zoechling; F Liebner; AJungbauer,*Food Funct*, **2011**, 2(1), 28-38.