# Antioxidant Activity And Hypoglycemic Potential Of Antidesma Ghaesembilla Gaertn (Phyllantaceae)

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**Abstracts:** The present study aimed to: establish a preliminary phytochemical profile and investigate the antioxidant and hypoglycemic potential of the crude methanolic extract of A. ghaesembilla Gaertn (Phyllantaceae). Limit test for acute toxicity (OECD 423) was performed on Sprague-Dawley rats which showed no mortality and is nontoxic up to 5000mg/kg.Scavenging activity revealed an IC5 0of 113 ppm (µg/mL) for DPPH radicals and IC50 estimates of 1724 µmol TEAC/100g DW for ABTS assay. Reducing power of the crude MEAG showed lower activity compared with the standard, Ascorbic acid. Hypoglycemic study showed a percentage decreased in mean glucose level of 56.65%, 55.06% and 54.47% following administration of the crude extract at doses of 400mg, 100mg and 1000mg/kg BW, respectively for 21 days. The data demonstrated that the effect of the extract is not dose-dependent.

**Keywords:** Antidesma ghaesembilla, Alloxan-induced diabetic rats, antioxidant activity, hypoglycemic, Limit Test, TEAC

## INTRODUCTION

The use of herbal and traditional medicines is a common practice as the option of traditional medicines in the treatment of lifestyle associated diseases such as hypertension and diabetes mellitus is found to be more practical due to its cheaper cost. Diabetes is a growing global epidemic as not only that diabetes is the fourth leading cause of death by disease globally[2], but also that is has become a major degenerative ailment as it carries with it complications of hypertension, atherosclerosis and microcirculatory disorders[3]. Diabetes is broadly classified into two categories, type 1 diabetes (insulin dependent or Juvenile) and type 2 diabetes (non-insulin dependent or T2DM). Type 1 – IDDM is caused by autoimmune-mediated destruction of cells of the islet of Langerhans, leading to total or near total insulin deficiency[4]. On the other hand, type 2 diabetes (T2DM) results from a combination of resistance to insulin action and an inadequate compensatory insulin secretion[5].


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Sadly, T2DM is increasing at an alarming rate globally and the Philippines is no exception to this as diabetes has affected around 4.6% (3.9 million) of Filipino population and the number is presumed to be doubled by 2030[6,7] These scenarios have driven people to seek alternative medicines which are believed to have lesser side effects as it is from natural sources. The search for new remedies is now directed toward the plant kingdom as active principles of many plant species are isolated for direct use as drugs, lead compounds, or pharmacological agents[12]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes[13,14] Among those plants used traditionally for the treatment of diabetic complications are plant species belonging to genus *Antidesma.* One of such plant is *Antidesma ghaesembilla* Gaertn (*Phyllanthaceae*), which has been asserted to provide various culinary and medicinal properties[16] . *Antidesma ghaesembilla* Gaertn is an indigenous species found in Ilocos Norte, Philippines. There are, however, no current studies in the Philippines on *A. ghaesembilla* Gaertn, concerning its efficacy on diabetes. The present work aimed to determine whether the methanolic leaf extract of *Antidesma ghaesembilla* Gaertn has hypoglycemic and antioxidant activities, and attempted to correlate these activities with the secondary metabolites present in the extract.

## EXPERIMENTAL SECTION

### Materials and Methods

#### Chemicals and Instruments

Analytical grade chemicals, chromatography materials and glucose assay kit has been obtained from Sigma-Aldrich Chemical Co. Spectrophotometric measurements have been accomplished using the equipment available at Thomas Aquinas Research Center (TARC), University of Santo Tomas.

#### Extraction Procedure

Fresh leaves of *A. ghaesembilla* Gaertn were collected, cleaned and air-dried at room temperature, cut into small pieces and then pulverized using a Wiley Mill. The powdered sample (165g) was used for the extraction process using absolute 1,650 mL by exhaustive percolation. The extract was concentrated under reduced pressure until

a viscous mass, known as crude extract was obtained and kept refrigerated until used for various phytochemical analysis, toxicity test and evaluation of the hypoglycemic property in rats.

**Phytochemical Screening by Thin Layer Chromatography (TLC)**

The TLC plates (precoated TLC plates Silica Gel G), cut to strips and marked by a straight line to indicate the position of the origin. The MEAG crude extract was spotted on the origin by using a capillary tube and placed in a lidded glass chamber containing a solvent system. The level of solvent in the lidded glass chamber was about 1 cm beneath the origin. The solvent travelled up the plate by capillary action till it reached the solvent front and was also marked by a straight line across in Silica gel (G) 60. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. They were later sprayed with different spraying reagents and were air-dried for few minutes for the development of color in separated bands. The movement of the analyte was expressed by its retention factor (Rf).

**Estimation of Total Phenolic Content (TPC) by Folin-Ciocalteu Method**

A stock solution (1mg extract: 1 mL MeOH) was prepared. 50 µL of the crude MEAG sample was taken and diluted in 1:4 ratio with DIH2O (200 µL) followed by 430 µL DiH2O and 20 µL Folin-Ciocalteu reagent. All were mixed and such mixture were allowed to react for 5 min before adding 50 µL of 20% Na2CO3, and 450 µL DIH2O. The reaction mixture was allowed to stand for 60 min at room temperature and absorbance was measured at 765 nm (Genesys 10 Spectrophotometer).The assay was measured in triplicate and results were averaged. Total phenolic content had been calculated using a calibration curve for gallic (10ppm-500ppm) on standard gallic acid graph. The concentration of total phenols was expressed as milligram gallic acid equivalent (GAE) per gram crude MEAG (mg/g of dry extract).

**Estimation of Total Flavonoid Content (TFC)**

The crude MEAG (1 mg) was mixed with 1 mL methanol, 1 mL of 2% Aluminum chloride in ethanol and a drop of acetic acid was added. The reaction mixture was then diluted in 25 mL ethanol and had been incubated for 30 minutes at room temperature before the absorbance was measured at 415 nm (Genesys 10 Spectrophotometer). Deionized water (1 mL) was used as reagent blank in place of the aluminum chloride solution. Three independent measurements were then performed and the averaged were then computed. The total flavonoid content was calculated using a calibration curve for quercetin as the standard. The result was expressed as milligram quercetin equivalent per gram crude MEAG (mg/g of dry extract).

**Ferric Chloride Test for Flavonoids, Tannins and phenolic Compounds**

**Test for free flavonoids**

0.1 g of the crude methanolic extract was dissolved in 2 ml ethyl alcohol and treated with few drops of ferric chloride solution. The result was a formation of blackish red or deep blue color, indicating the presence of flavonoids.

**Test for Phenolic compounds**

To test for phenolic compounds, a 2 ml of filtered solution of the methanolic crude extract of the plant material, three drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferricyanide were added to detect phenolic compounds. The formation of bluish-green color meant that the result was positive.

**Test for tannins**

Two milliliters of the methanolic crude extract solution was added to few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue color showed the presence of gallic tannins and a green- blackish color indicated the presence of catechol tannins.

**Antioxidant Assays**

**Antioxidant Screening with TLC- DPPH Method**

The crude MEAG extract with appropriate dilution was spotted on the silica gel coated plate and developed in a tank containing the mobile phase (Ethanol: Hexane , 6:4). The plate was then dried and observed under UV 366 nm for spot/band formation. Methanolic solution of DPPH (0.2%) was sprayed on the plate and the plate was left to dry. The coloration produced on the plate was noted. Positive result showed a yellow colored spot on a purple background.

**Free-Radical Scavenging DPPH (2, 2-diphenyl-1-picrylhydrazy) Assay**

For the DPPH assay, the stable DPPH radical was used to measure the free radical scavenging activity of the crude MEAG sample extract as described by Habib *et al*., in 2011, with minor modifications. The reaction mixture consisting of crude MEAG extract in varying concentrations (16.625 – 500 ppm) and 1 mL of freshly prepared DPPH solution (0.004 g/mL) was mixed and was allowed to incubate in the dark at room temperature. After which, the absorbance was then measured at 517 nm using a UV-vis spectrophotometer after 30 minutes. Methanolic DPPH solution and deionized water served as the blank control and was assayed in the same condition. Three independent measurements were performed and results had been averaged. The free radical-scavenging activity (FRSA) was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where Acontrol was the mean absorbance of the methanolic DPPH solution and Asample was the mean absorbance of particular concentration of the crude extract solution in the actual determination. The antioxidant activity of the crude MEAG extract was expressed as IC50

(inhibition concentration) of the crude extract required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as standard. The inhibition curve was also plotted and IC50 values were then calculated.

**ABTS *TEAC Assay**

A stock solution containing 7 mM ABTS solution and 2.45 mM potassium persulfate (final concentration) solution was prepared and were then equally mixed and allowed to stand for 12-16 hours in the dark at room temperature. Oxidation of the ABTS was commenced immediately, but the absorbance did not become stable until more than 6 hours had elapsed. The radical cation had then been found to be stable in this form for more than 2 days in storage, in the dark at room temperature (Re *et. al*., 1999). The crude MEAG extract (50 µL) were allowed to react with 130 µL of ABTS solution and were mixed thoroughly. The reaction mixture was incubated for 6 mins before the absorbance was measured at 734 nm using UV-vis spectrophotometer. Trolox was used as reference standard (final concentration 5-25 µM) in 80% ethanol and was assayed in the same condition. The result was then expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µmol Trolox equivalent per 100 g dry weight of plant). Three independent measurements were performed and results had been averaged. The percentage inhibition was computed using the following formula:

$$\% \text{ inhibition} = \frac{(1 - \text{Abs test sample})}{\text{Abs blank}} \times 100$$

Where Abs test sample was the mean absorbance of particular concentration of the crude extract in the actual determination and Abs blank was the mean absorbance of ABTS in Ethanol.

**Reducing Power**

The crude MEAG at various concentrations was added to 1 mL of distilled water. Phosphate buffer (2.5mL, 0.2M, pH 6.6) and potassium ferricyanide ($K_3$ Fe (CN) $_6$ ) (2.5mL, 1%) was mixed with the aqueous crude extract. The resulting reaction was incubated at room temperature for 30 minutes. To the mixture, 2.5 mL of trichloacetic acid (10%) was added, and the same was placed in a centrifuge at 3,000 rpm for 15 minutes. After which, the upper layer of the solution (2.5mL) was taken and mixed with 2.5 mL distilled water and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm. The reference standard used was ascorbic acid, while phosphate buffer (pH) had served as the blank standard. Three independent experiments had been performed and expressed as mean +SD.

***In Vivo* Studies**

**Hypoglycemic studies**

The crude extracts were reconstituted in appropriate amount of solvent (5% propylene glycol in NSS) depending on the dose desired for the acute toxicity test. In vivo hypoglycemic studies required Alloxan-induced diabetic rats to be orally administered with the MEAG crude extract for 21 days. Fasting blood glucose level had been taken before Alloxan induction, and had been recorded as the baseline glucose level of the rats. Every week, starting on the 1st, 7th, 14th and 21st. fasting blood glucose was monitored by drawing blood samples from the tip of the tail and was measured using the glucose assay kit (Glucose Liquicolor; Sigma Aldrich).

**Acute Toxicity Test (Limit Test)**

The limit test was employed as the test material was likely to be non-toxic, based on the information gathered by the researcher. Six female Sprague-Dawley rats (130-175 g) 60 days old had been employed in this study. Normally females were used[20] because literature surveys of conventional LD50 tests showed although there was little difference in sensitivity between the sexes, in those cases where differences were observed females were generally slightly more sensitive[21]. The animals were selected randomly, grouped into two groups (A-B) of three rats per group, marked for proper identification and were kept for at least 5 days in caged for laboratory acclimatization prior to the procedure. Animals were fasted (food, but water was not withheld) overnight (12h) prior to dosing. The fasted weight of each rat had been determined before giving the dose. A limit test at one dose level of 2000 mg/kg BW was given to Grp A, three animals. The animals were observed continuously for the first 4h (at least once during the first 30 minutes) and for the next 6, 24, and 48h after extract administration and daily thereafter, for a total of 14 days. The researcher opined that if no death will occur, a limit test at one dose level of 5000 mg/kg was given to another group of three animals (Grp B) on the 5th day, following the procedure done on group A. Neurological and behavioral changes were observed for any signs of acute toxicity and recorded[20] . All mortality within a period of testing the extract was also noted.

**Alloxan-induced diabetic rats**

Sprague-Dawley rats of both sexes (120-150g) were obtained from DOST and were then acclimatized for 5 days to 25 ℃, 50% humidity, and 12 h dark/light cycle with food and water. The animals were weighed individually prior to Alloxan-induction. Alloxan monohydrate was dissolved in sterile distilled water and was then administered at the dose of 120mg/kg BW[22]by a single intraperitoneal injection in overnight fasted (12h) rats. After 72h, the fasting blood sugar level was checked. The rats with elevated fasting plasma glucose above 240 mg/dL were chosen for this study. The rats were then assigned into six groups of 6 rats (both sexes) which are as follows:

Group I - Negative control (Normal Rat with Fasting plasma glucose<5.9 mmol/L, 107
mg/dL) received PEG (propylene glycol, solvent that was used to dissolve the
MEAG extract) 5 mL/kg BW
Group II - Diabetic control, received the PEG 5 mL/kg BW
Group III – Diabetic control, received the MEAG extract at the dose of 100 mg/ kg BW
Group IV - Diabetic control, received the MEAG extract at the dose of 400 mg/kg BW
Group V - Diabetic control, received the MEAG extract at the dose of 1000 mg/kg BW
Group VI - Positive control, received the Glibenclamide (standard drug) 5 mg/kg BW

All were administered by oral gavage. The blood glucose levels and the weight of the rats were estimated at 0, 7th, 14th, and 21th with the drug and extract being administered daily for 21 days from the day of induction. Blood samples were drawn from the tip of the tail and were measured and the results were then compared with those of the 6th group treated with glibenclamide (5 mg/kg BW), a standard oral hypoglycemic agent[23]. All procedures in the experiments were guided by the strict observance of animal ethics guidelines, Institutional Animal Care and Use Committee (IACUC no. RC2013-240712; 09/05/2013) and with permissions from the Research Center for the Natural and Applied Sciences-UST (RCNAS).

### Statistical Analysis

Percent yield of extraction process was expressed as mean + standard error (SEM). Means and its standard error (SEM) were used to summarize the data gathered from the experiment. Linear regression analyses were performed in the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), while non-linear regression was used to estimate DPPH, Reducing power and ABTS IC50. Moreover, Repeated Measures Analysis of Variance was used in the hypoglycemic activity and weights of the rats. All the statistical tests, at 5% level of significance, were performed using SPSS 20.0 and Prism 6.0.

## RESULTS AND DISCUSSIONS

### Plant extraction and Phytochemical Screening of Secondary Metabolites

165 g leaves of *A. ghaesembilla* Gaertn in triplicate analysis produced a w/w average yield of 15.36% + 0.20169w/w. Table 4 shows the individual yields obtained from each trial. At 95% confidence interval, mean percent yield under the same extraction process is from 15.16 % to 15.56 % w/w. The TLC analysis of the crude MEAG and Ethanol: Hexane (6:4) solvent system vividly showed the presence of spots of different phytoconstituents.

**Table 1.** SUMMARY OF RESULTS (TLC with Spray Reagents)

| SPRAY REAGENT | CONSTITUENTS TESTED | INFERENCE |
|---|---|---|
| Potassium Ferricyanide -Ferric chloride | Phenols, Tannins, Flavonoids | ++ |
| Dragendorff's reagent | Alkaloids | – |
| Van-Urk Salkowski | Indoles | – |
| Vanillin-Sulfuric acid | Higher alcohols, phenols, steroids, Essential oils | + |
| α – napthol-sulfuric acid | Sugars | – |
| Borntrager reagent | Coumarins, Anthraquinones, Anthrones, phenols | + |

+ Present; ++ strongly present; - Absent

### Results of Ferric Chloride Test

The Ferric chloride test for phenols, tannins and flavonoids revealed positive results of blue, blackish-green and blackish-red color, respectively[25] .

**Table 2.** Summary of Results: Ferric Chloride Test

| Ferric chloride | VISIBLE RESULT | |
|---|---|---|
| Crude MEAG | Blackish color with red tint | + Flavonoid |
| | Bluish-green color | + Phenol |
| | Blackish- green color | + Catechol Tannin |

### *In vitro* Antioxidant Capacity

The crude MEAG extract displayed moderate to strong antioxidant activity relative to Ascorbic acid, which is a pure compound; however it exhibited a lower antioxidant capacity as equivalent to trolox in ABTS assay.

### Total Phenolic Content (TPC)

The total phenolic content of the crude extract based on net mean absorbance reading of 0.628 (Table 3) was found to be 430.5 mg gallic acid equivalents(GAE) per gram of crude extract in reference to the standard gallic acid curve (y = 0.0012, x = + 0.1114, $R^2$ = 0.9334).

**Table 3.**Total Phenolic Content Determination (Crude MEAG Extract)

| Trial 1 | Trial 2 | Trial 3 | Average |
|---|---|---|---|
| 0.653 | 0.643 | 0.588 | 0.628 |

Samples were evaluated at 500 ppm

### Total Flavonoid Content (TFC)

The total flavonoid content of the crude MEAG extract based on net absorbance reading of 0.162 (Table 8) was 22.89 mg quercetin equivalent (QE) pergram of crude extract in reference to the standard quercetin curve (y = 0.0059;x = 0.0269; $R^2$ = 0.9728 ).

**Table 4** Total Flavonoid Content determination (Crude MEAG extract)

| Trial 1 | Trial 2 | Trial 3 | Average |
|---|---|---|---|
| 0.150 | 0.126 | 0.209 | 0.162 |

Samples were evaluated at 500 ppm.

### Free-Radical Scavenging by TLC- DPPH Method

TLC based DPPH spray method was used for evaluation of qualitative method antioxidant activity of the crude MEAG extract. The change in coloration observed in the plates from purple to yellow in a purple background showed that the crude MEAG extract possess free radical scavenging activity and active antioxidant property[30] .

### DPPH Assay

An examination on the DPPH scavenging activity shows a concentration response relationship in the crude MEAG

extract. An increase in concentration is also equivalent to an increase in scavenging capacity. This means that at lower concentration the MEAG extract showed lower inhibitory capacity as compared with the standard Ascorbic acid. But as the concentration of the extract increased, activity also remarkably increased and was found to have higher inhibitory result at 125ppm and 250ppm compared with Ascorbic acid. IC50 values were obtained from plotted graph of % inhibition activity and were found to be 89.7 ppm (CI95%: 64 to 126 ppm, $R^2$ = 0.906) for Ascorbic acid and 113 ppm (CI95%: 84.6 to 151 ppm, $R^2$ = 0.918) for the crude MEAG extract based on the Four-parameter logistic regression shown in Figure 1 and Table 5.

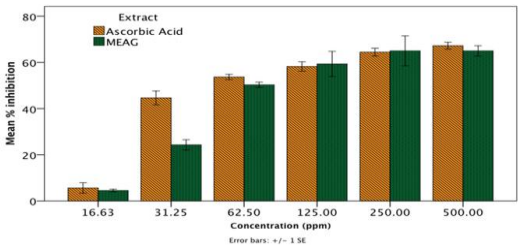


**Figure 1.** DPPH: Graphical Representation of the Mean % inhibition vs Different Concentrations of AA and MEAG extract

**Table 5.** IC50 estimates For AA and crude MEAG

| Test Samples | $IC_{50}$ estimate | $R^2$ |
|---|---|---|
| Ascorbic Acid | 89.7 (64 to 126) | 0.906 |
| MEAG | 113 (84.6 to 151) | 0.918 |

Values expressed as $IC_{50}$ estimate (95% confidence intervals in parentheses

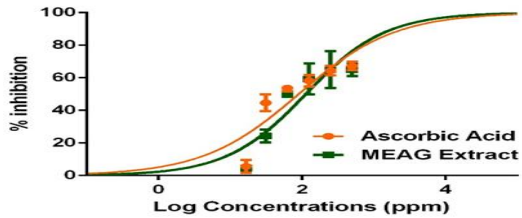


**Figure 2.** Four Parameter Logistic Regression Graph for DPPH

The four-parameter logistic regression showed that the IC50 estimate for ascorbic acid is 89.7 ppm (CI95%: 64 to 126 ppm, R2 = 0.906) and for MEAG is 113 ppm (CI95%: 84.6 to 151 ppm, R2 = 0.918).

## ABTS Assay

In ABTS radical cation scavenging method, the activity of the crude MEAG extract was expressed as Trolox equivalent micromolar (µMol) of Trolox solution having an antioxidant capacity equivalent to 100 g dry weight of plant. The antioxidant capacity of the crude MEAG extract ranged from 0.015 µmol TEAC to 3117.5 µmol TEAC, for 62.5 ppm and 1000ppm, respectively from lowest to highest concentration. TEAC values increased as the concentration also increases (Table 6). The four-parameter logistic regression has estimated the following IC50: 19.93 ppm (CI95%: 16.7 to 23.8, R2 = 0.894) for Trolox; and 1724 ppm (CI95%: 1302 to 2282, R2 = 0.989) for MEAG, shown in Figure 3.

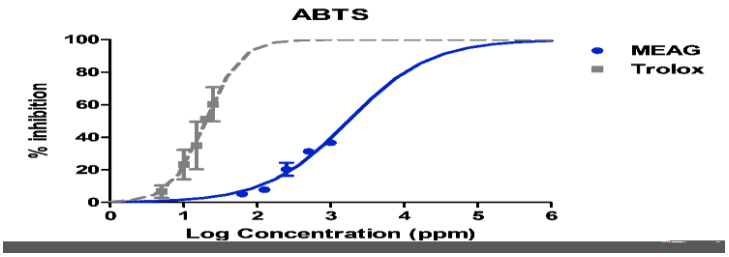


**Figure 3.** Four Parameter Logistic Regression Graph for IC50 of MEAG and Trolox

## Reducing Power Assay

The crude MEAG extract caused the reduction of the $Fe^{+3}$/ferricyanide complex to the ferrous form, which can be observed by examining the formation of a Prussian blue color at 700 nm. Figure 4 showed the dose response bar graph for the reducing power of the extract. The crude MEAG extract when compared with the standard Ascorbic acid showed lower activity. The data demonstrated at Fig. 4, however, disclosed that the extract was moderately strong, while little increment and almost a steady state condition was observed when concentration was increased. Table 6 shows the analysis of variance in the result.

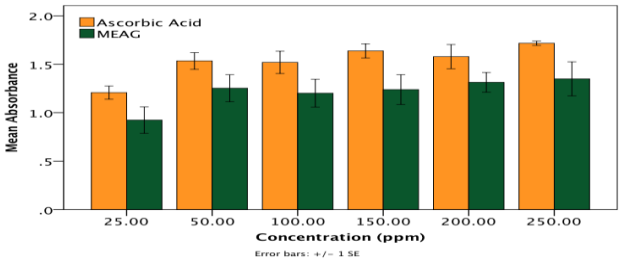


**Figure 4.** Reducing Power ability of the crude MEAG vs Ascorbic Acid at Different concentrations.

**Table 6.** Reducing Power: Analysis of Variance Result

| Source of Variability | F stat | p-value |
|---|---|---|
| Samples*Concentration | 0.101 | 0.991 |
| Samples | 21.537 | <0.001 |
| Concentration | 3.675 | 0.013 |

Analysis of Variance shows that the interaction effect of the test samples (standard and extract) and concentrations are not significant [F5, 24=0.101, p=0.991] indicating that the mean absorbance of the test samples do not differ according to concentrations. The mean absorbance of the two samples (standard and extract) significantly differ [F1, 24 = 21.537, p<0.001] in which it shows that the mean absorbance of the ascorbic acid, regardless of concentrations, is greater than the crude MEAG. On the other hand, the mean absorbance of the different

concentrations, regardless of the sample, significantly differ [F5, 24=3.675, p=0.013], post hoc analysis [Tukey's HSD: 25 ppm < (50 = 100 = 150 = 200 = 250 ppm)] showed that the mean absorbance of the 25 ppm is significantly the least, while other concentrations (50, 100, 150, 200 and 250 ppm) do not differ (p=0.206).

### *In vivo* Studies on Rats

**Acute toxicity study**

It was found that the animals were safe up to a maximum dose of 5,000 mg/ kg body weight, according to acute oral toxicity studies using the limit test method (OECD 423). There were no changes in normal behavior pattern and there were no observed signs and symptoms of toxicity and mortality.

### Acute Toxicity Factsheet for Crude MEAG Extract

**Table 7A.** LIMIT TEST: Animals tested with 2000 mg/ kg BW crude MEAG extract

| Group A (3-Animals ) and their acute oral toxicity study detailed initials | | | |
|---|---|---|---|
| ID No.Experi-mental Animals | Body Weight | Extract Used at 2000mg/ kg body wt. | Dose Prepared In 5% PEG in NSS |
| SD R-1 2013 | 160 g | 320 mg | 320mg/0.94 mL |
| SD R-2 2013 | 170 g | 340 mg | 340mg/1mL |
| SD R-3 2013 | 156 g | 312 mg | 312mg/0.92mL |

**Table 7B.**LIMIT TEST: Animals tested with 5000 mg/ kg BW crude MEAG extract

| Group B (3-Animals) and their acute oral toxicity study detailed initials | | | |
|---|---|---|---|
| ID No. Experimental Animals | Body Weight | Extract Used at 5000mg/kg body wt. | Dose Prepared In 5% PEG in NSS |
| SD R-1 2013 | 166 g | 830 mg | 830mg/1mL |
| SD R-2 2013 | 148 g | 740 mg | 740mg/0.89mL |
| SD R-3 2013 | 146 g | 730 mg | 730mg/0.88mL |

**Hypoglycemic Activity**

Administration of single intraperitonial injection of 120 mg/ kg BW of freshly prepared Alloxan monohydrate produced diabetes in Sprague-Dawley rats after 72 hours. The induction of Alloxan with the experimental groups (Group 2 – 6) produced diabetic rats as shown in Table 8.

**Table 8.** Glucose Levels from Baseline to Day 1

| Groups | Baseline | Day 1 | p-value |
|---|---|---|---|
| Group 1: Control Group | 71.23 ± 6.40 | 97.58 ± 3.08 | 0.003 |
| Group 2: DIABETIC RATS on PEG 5ml/kg | 68.06 ± 7.25 | 591.86 ± 84.00 | 0.002 |
| Group 3: DIABETIC RATS with 100mg/kg MEAG extract | 85.77 ± 5.35 | 688.80 ± 62.05 | <0.001 |
| Group 4: DIABETIC RATS with 400mg/kg MEAG extract | 79.89 ± 8.72 | 297.19 ± 14.95 | <0.001 |
| Group 5: DIABETIC RATS with 1000mg/kg MEAG extract | 100.09 ± 8.62 | 297.68 ± 29.21 | <0.001 |
| Group 6: Positive control with GLIBENCLAMIDE 5mg/kg | 85.27 ± 3.92 | 334.41 ± 32.12 | <0.001 |

Values expressed as mean ± SEM, n = 6.
P-value is based on paired t-test.

Based on the data shown in Table 10 , among the doses of the crude MEAG extract, 400 mg/ kg BW (Group 4) was the effective dose that lowered the mean blood glucose by 56.65 % (+ 6.11) at day 21, followed by 100mg/kg BW (Group 3) at 55.06% (+ 8.12) and 1000mg/kg BW (Group 5) at 54.47 (+ 6.46). Treatment of the 6th group with the

**Table 9.**Effects of Different Doses of Crude MEAG Extract on Blood Glucose Levels (mg/ dL) of Diabetic Rats at Weekly Intervals from Week 1-3

| **Groups** | **Day 1 (0)** | **Day 7 (1st)** | **Day 14 (2nd)** | **Day 21(3rd)** | **p-value** |
|---|---|---|---|---|---|
| Group 1: Control Group | 97.58 ± 3.08 | 88.14 ± 3.31 | 99.48 ± 1.87 | *42.38 ± 5.39 | <0.001 |
| Group 2: DIABETIC RATS on PEG 5ml/kg | 591.86 ± 84.00 | 477.90 ± 63.30 | 415.28 ± 42.04 | *235.51 ± 36.21 | <0.001 |
| Group 3: DIABETIC RATS with100mg/kg MEAG extract | 688.80 ± 62.05 | 608.04 ± 70.28 | *364.51 ± 68.87 | *306.03 ± 53.77 | <0.001 |
| Group 4: DIABETIC RATS with400mg/kg MEAG extract | 297.19 ± 14.95 | *268.00 ± 12.74 | *261.49 ± 11.08 | *132.75 ± 24.17 | <0.001 |
| Group 5: DIABETIC RATS with 1000mg/kg MEAG extract | 297.68 ± 29.21 | 284.53 ± 29.26 | *272.44 ± 26.38 | *136.18 ± 21.21 | <0.001 |
| Group 6: Positive control with GLIBENCLAMIDE 5mg/kg | 334.41 ± 32.12 | *251.68 ± 39.79 | *215.54 ± 52.08 | *84.25 ± 10.81 | <0.001 |

Values expressed as mean ± SEM, n = 6.
P-values are based on Repeated Measures ANOVA.

*p<0.05, compared with the glucose level at day 1.

**Table 10.** Weights of Rats from Baseline to day 21

| Groups | Baseline | Day 1 | Day 7 | Day 14 | Day 21 | p-value |
|---|---|---|---|---|---|---|
| Group 1: Normal Rats on PEG 5 mL/Kg | 135.00 ± 3.03 | 134.00 ± 2.62 | *162.17 ± 3.79 | *182.50 ± 5.12 | *192.17 ± 7.66 | <0.001 |
| Group 2: Diabetic Rats on PEG 5 mL/Kg | 139.50 ± 6.54 | 135.83 ± 6.66 | 150.83 ± 6.51 | *168.33 ± 9.46 | 159.50 ± 6.10 | 0.003 |
| Group 3: Diabetic Rats with 100 mg/Kg MEAG extract | 195.50 ± 6.87 | 185.83 ± 7.35 | 193.67 ± 7.74 | 195.33 ± 8.52 | 198.67 ± 8.62 | 0.137 |
| Group 4: Diabetic Rats with 400 mg/Kg MEAG extract | 137.33 ± 5.97 | 132.33 ± 7.99 | 151.67 ± 11.67 | *170.17 ± 13.60 | *183.83 ± 11.96 | <0.001 |
| Group 5: Diabetic Rats with 1000mg/Kg MEAG | 144.00 ± 4.65 | 139.17 ± 6.18 | *153.33 ± 6.14 | *169.00 ± 7.66 | *180.00 ± 8.06 | <0.001 |
| Group 6: Positive Control – Diabetic Rats with Glibenclamide 5mg/Kg BW | 137.00 ± 4.07 | 144.50 ± 4.11 | *159.83 ± 4.13 | *193.00 ± 11.90 | *215.67 ± 14.73 | <0.001 |

Values expressed as mean ± SEM, n = 6.
P-values are based on Repeated Measures ANOVA.

The data (Table 10) revealed the weight increased in the groups of diabetic rats at weekly intervals after treatment with the crude MEAG extract and the standard drug, Glibenclamide for Group 6. This observation also discloses that the crude MEAG extract might possibly have protective effect on the body weights of diabetic rats due to its ability to reduce and control blood glucose level.

### Correlation Studies

Relationship between the Antioxidant Activity and Total Phenolic Content

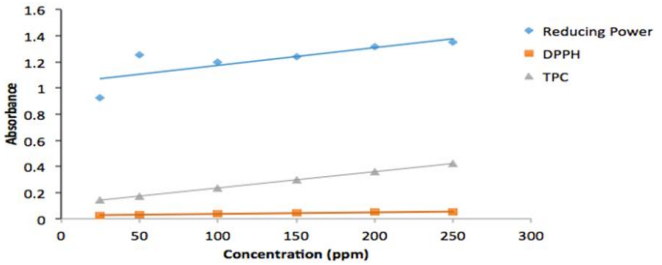


**Figure 5.** Graphical Representation of the Correlation Coefficients of Reducing Power, DPPH, and TPC

**Table 11.** Correlation Coefficient Values of Reducing Power, DPPH, and TPC

| | | | |
|---|---|---|---|
| Reducing Power with DPPH | 11.258 | 0.779 | 0.034 |
| Reducing Power with TPC | 1.091 | 0.779 | 0.034 |
| TPC with DPPH | 10.321 | 1.000 | <0.001 |

Correlation coefficients indicate that DPPH (R=0.779, p=0.034) and TPC (R=0.779, p=0.034) are positively correlated with reducing power. In addition, an increase in one unit of absorbance in DPPH and TPC will lead to an increase of 11.258 and 1.091 units in the reducing power. On the other hand, TPC is positively correlated (R=1.000, p<0.001) with DPPH and further, an increase of one unit of absorbance in DPPH leads to an increase of 10.321 in the absorbance of TPC. The data showed (Figure 16 & Table 23) that the over-all relationship between the total phenolic content and the antioxidant activity of the crude MEAG extract was a positive and highly significant linear correlation. The statistical result suggested that the presence of phenolic compounds in the crude MEAG extract contributed significantly to its antioxidant capacity. Correlation Analysis between Hypoglycemia and DPPH (Hypoglycemic Activity and Antioxidant Capacity)

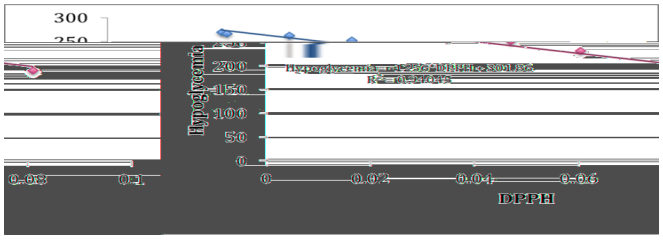


**Figure 6.** Graphical Representation of the Correlation between Hypoglycemia and DPPH

DPPH, likewise, is negatively correlated (R = -0.97, p=0.001) to hypoglycemia, in which one unit of increase in DPPH leads to 1256 units of decrease in hypoglycemia. This negative linear correlation prove that increased in hypoglycemic effect(in terms of fall in blood glucose level of rats) of the crude MEAG extract shows lower antioxidant activity (in terms of free radical scavenging activity by DPPH),while increased in antioxidant activity results to decreased hypoglycemic capacity of the crude MEAG extract.

## CONCLUSION

In view of the results generated from this study, the researcher therefore concluded that the methanolic leaf extract of *Antidesma ghaesembilla* Gaertn contains secondary metabolites which are associated with important biological activities. The crude extract has moderate to strong antioxidant potential owing to the presence of polyphenolic compounds, and the ability to scavenge free radicals. It possesses significant hypoglycemic potential as it reversed the fasting blood sugar of diabetic rats to near normalcy. It is also found to be of similar effectiveness to Glibenclamide 5 mg, an existing oral hypoglycemic drug in terms of blood glucose reduction. Due to the shown efficacy of the crude methanolic leaf extract of *A. ghaesembilla*, further studies on the plant's potential medical value is highly warranted.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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