



Three Cagayan Island endemic flora: Its phytochemicals and hypoglycemic effect on alloxan induced Sprague Dawley Rat

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Abstract

Cagayan province is blessed with vast flora with presence of many island species that are endemic. Currently, different botanicals have been formulated as pharmaceutical agents to developed new products essential for human wellbeing. This study generally aimed to determine the secondary metabolites present in the three island endemic plant species in Cagayan namely; Igem dagat (*Podocarpus costalis* C. Presl), Balingagta (*Drypetes falcata* Pax & K. Hoffm.) and Babuyan lunas (*Lunasia babuyanica* Merr.) and to determine their hypoglycemic effect on Sprague Dawley Rats. Ethanolic leave extract of the three endemic plant species were used and tested for its phytochemicals. Complete randomized design (CRD) was used for the hypoglycemic potential with three replications for each species and Alloxan was used to induce hyperglycemia in the rats. The results reveals that *P. costalis*, *D. falcata* and *L. babuyanica* contains alkaloids, flavonoids, glycosides, tannins and triperthenes. Likewise, *L. babuyanica* and *D. falcata* were also positive with saponin and phytosterols. Analysis of variance (ANOVA) for the initial glucose level, after Alloxan induced and after treatment of plant extracts did not show significant difference across treatment means. Among the treatments, T₃ (*L. babuyanica* ethanolic leave extract) significantly reduced the Alloxan induced hyperglycemia in Sprague Dawley Rats. Glucose level were reduced with the administration of the other treatments but not significant to reject the null hypothesis. All the plant extracts have hypoglycemic effect however, T₃ (*L. babuyanica* ethanolic leave extract) is more effective.

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Introduction

Situated within the Cagayan Valley region, the province is bounded by the Philippine Sea on the east; on the south by Isabela province; on the west by the Cordillera Mountains; and on the north by the Balintang Channel and the Babuyan Group of Islands. About 2 kilometres (1.2 mi) from the northeastern tip of the province is the island of Palau; a few kilometers to the west is Fuga Island. The Babuyan Group of Islands, which includes Calayan, Dalupiri, Camiguin, and Babuyan Claro, is about 60 nautical miles (110 km) north of Luzon mainland. This island comprises a unique flora that most of the islanders used them in their traditional healing practices. Three of these plants are endemic in the island town of Calayan. *The three island endemic plant species in Cagayan namely; Igem dagat (Podocarpus costalis C. Presl), Balingagta (Drypetes falcata Pax & K. Hoffm.) and Babuyan lunas (Lunasia babuyanica Merr.).*

Plants are richest source of bioactive secondary metabolites in a most effective way and with specific selectivity. From the start of human being development men were using different medicinal plants as traditional medicines for their health care. Plants have the ability to produce several valuable classes of chemical constituents which showed interesting biological action.

Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from its various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents. They serve as a raw material base for elaboration of more complex semi-synthetic chemical compounds. Due to these active compounds that are present in plants many diseases have been treated, like hyperglycemia.

Hyperglycemia is associated with high blood glucose level. Severely elevated glucose levels can farther

result in a medicinal emergency like diabetic ketoacidosis (DKA) or hyperglycemic hyperosmolar nonketotic (HHNS) also referred to as hyperglycemic hyperosmolar state). The main symptoms of hyperglycemia are increased thirst and a frequent need to urinate.

Objectives of the Study

This study aimed to determine the phytochemical constituents and hypoglycemic effect of the three Cagayan Island endemic plant ethanolic extract on Sprague Dawley Rats (*Mus musculus*).

Specifically, this study sought to determine the following:

1. The phytochemical constituents present on the three Cagayan island endemic plant ethanolic extract namely: Igem dagat (*Podocarpus costalis C. Presl*), Balingagta (*Drypetes falcata Pax & K. Hoffm.*) and Babuyan lunas (*Lunasia babuyanica Merr.*);
2. The percent decrease of the blood glucose level of Sprague Dawley Rats after fasting and after administration of the different treatments; and
3. The treatment with the highest lowering effect on the blood glucose level of the experimental rat

Materials and methods

Materials

The materials that were used in the study were sterilized glass container, beakers, test tubes, dropper, cheese cloth, pipettes, weighing scale, well ventilated standard polycarbonate cages with mesh top and husk bedding, syringe, glucose strips, one touch electronic glucometer, gavage needle, metformin, rats, standard pellet diet and top water.

Plant Material

Leaves of three island endemic wild plants of Cagayan collected at Calayan Island namely,

1. Igem Dagat (*Podocarpus costalis C. Presl*), locally known as Arius, is a species of conifer in the family Podocarpaceae. It is endemic to the Philippines.
2. Balingagta (*Drypetes falcata Pax & K. Hoffm.*), Malpighiales belonging to the family of

Putranjivaceae an endemic species in Camiguin Island and Babuyanes

3. Babuyan Lunas (*Lunasia babuyanica* Merr.), Sapindales of the family Rutaceae. Endemic species in Camiguin Island, Philippines

Experimental Design and Treatment

The study was done to determine the hypoglycemic effect of the three plant leaves ethanolic extract. For the hypoglycemic study, Complete Randomized Design (CRD) was utilized with ten replicates per treatment. Four treatments were used in this study namely:

- T1 - distilled water (negative control)
- T2 - Babuyan Lunas ethanolic leaves extract
- T3 - Balingagta ethanolic leaves extract
- T4 - Igem Dagat ethanolic leaves extract

Collection of Samples

The samples used in this study were selected thoroughly by choosing healthy leaves and not infected from any diseases. The experimental animals were purchased in a certified laboratory for laboratory animal utilization. They are assigned in their proper groups and cages.

Preparation of Extracts

The leaves were washed thoroughly to remove dirt and impurities. One hundred grams of each plant leaves were powdered and placed in 100ml of 80% ethanol and was soaked for 48 hours. The extract was purified by removing the alcohol using a rotary vap. The collected extract was temporarily placed in a clean glass container and was kept in a refrigerator until used.

Administration

The 40 test animals were housed in a cage measuring 30Wx36Lx15H cm. Body weight in grams was taken from each animal. They were randomly grouped into 4 according to the treatment assignment. Tails are marked with permanent ink dot for identification numbers. Initial blood glucose was individually taken using the tail blood as sample. The test animals were held manually and the tail tip are cut with surgical scissor. The initial blood glucose from each mouse was assigned with their normative values and was

recorded. Another blood glucose was taken from the test animal after 4 hours of fasting (without food and water). It was recorded and compared with the initial blood glucose. The animals were fed with their regular diet and the different treatment was given by gavages' method. The blood sugar was again taken 24 hours after giving the assigned medication with the test animals on a fasting state. For T1 (negative control) distilled water. T2 50ml/KBW of Igem Dagat ethanolic extract was administered. T3 50ml/KBW of Balingagta ethanolic extract was administered. For T4 50ml/KBW of Babuyan Lunas was administered. They have been administered through oral gavages. After 24 hours of administration of metformin and extracts, blood glucose level (BGL) was taken.

Data Collection

The body weights of each test animal are measured during fasting. Likewise, the blood glucose levels of the test animals were also measured before and after administration of the different treatments. The mice were fed with pigeon pellets amounting to five percent of their total body weight in grams. Blood glucose levels are measured using digital glucometer 24 hours after the administration of the treatments and recorded it as fasting blood glucose levels after treatments.

Data Analysis

The data gathered in this study was analyzed using T-test and Analysis of Variance (ANOVA).

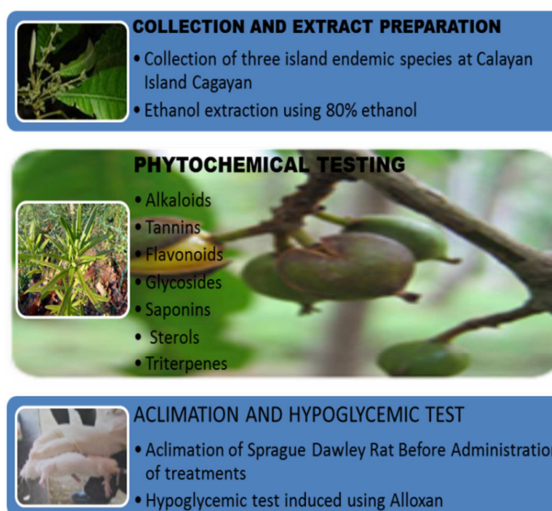


Fig. 1. General Flow Chart.

Analysis of variance (ANOVA) is used to uncover the effects of the treatment on the blood glucose level (BGL) of the test animals. Least Significant Difference (LSD) test was used in exploring the difference between treatments.

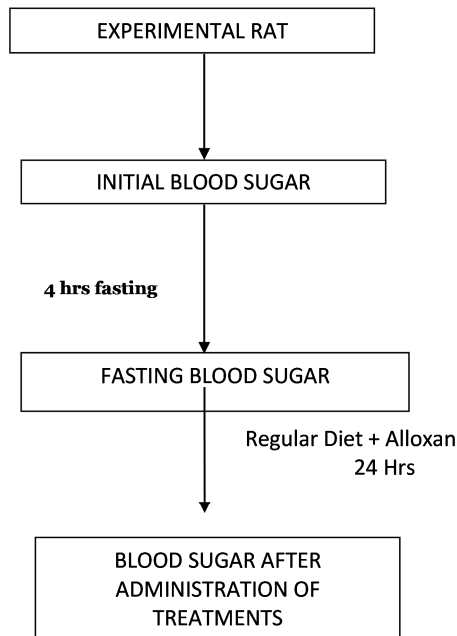


Fig. 2. Blood Sugar Monitoring.

Results and discussion

Table 1 shows the result of the phytochemical analysis of the three Island endemic plants species of Cagayan. It reveals that *P. costalis*, *D. falcata* and *L. babuyanica* contains alkaloids, flavonoids, glycosides, tannins and triperthenes. Likewise, *L. babuyanica* and *D. falcata* were also positive with saponin and phytosterols. Table 2 shows the percentage decrease of the fasting blood sugar from the initial sugar in the different treatments after 3 days. All the treatments show a decrease in blood sugar. Treatment 1 registered the highest decrease in blood sugar with 38.76% followed by T3, T4 and T2 with 31.10%, 24.30% and 12.68 % respectively. T-test between the initial and fasting blood sugar reveals that T2 and T3 are not significant and T1 and T4 are significant.

Table 3 on the other hand, shows the analysis of variance of the treatment means of the initial and fasting blood glucose. It reveals that the different treatment means are not significantly different with one another with observed value of 0.9814. This means that the percentage decreases in blood sugar after fasting are comparable among the treatments.

Table 1. Summary of Phytochemical Screening of Ethanolic Extract of the Three Island Endemic Plants.

| Parameters | Indication For Positive Result | Results | | |
|-------------|--------------------------------|------------------------------------|-----------------------------------|---|
| | | <i>P. costalis</i> (Igem Dagat) | <i>D. falcata</i> (Balingagta) | <i>L. babuyanica</i> (Babuyan Lunas) |
| Alkaloids | White/Cream precipitate | (+) | (+) | (+) |
| Flavonoids | Crimson Colour | (+) | (+) | (+) |
| Glycosides | Brick Red | (+) | (+) | (+) |
| Saponins | Honey Comb (froth formation) | (-) | (+) | (+) |
| Phytosterol | Blue Colour | (-) | (+) | (+) |
| Tannins | Brownish Colour | (+) | (+) | (+) |
| Triterpenes | Red/Yellow Colour | (+) | (+) | (+) |

*Legend: (-) negative, (+) positive

Table 2. T-test and percentage decrease of sugar of the initial blood sugar and the fasted blood sugar of the different treatments.

| Treatment | Initial Blood Sugar | Fasting Blood Sugar | % decrease | T-test | Decision |
|---|---------------------|---------------------|------------|-------------|----------|
| T1 Distilled water | 143.7 | 88 | 38.76 | 2.863043789 | * |
| T2 <i>L. babuyanica</i> Ethanolic Extract | 126.1 | 110.1 | 12.68 | 1.135876475 | ns |
| T3 Extract <i>D. falcata</i> Ethanolic | 128.6 | 88.6 | 31.10 | 2.544566789 | * |
| T4 <i>P. costalis</i> Ethanolic Extract | 125.1 | 100.8 | 24.30 | 1.135876475 | ns |

Table 3. Analysis of Variance of the initial blood sugar and the fasting blood sugar of the different treatments.

| Source of Variation | df | Sum of Squares | Mean Square | Observed F | Tabular F | |
|---------------------|----|----------------|-------------|----------------------|-----------|---------|
| | | | | | 5% | 1% |
| Treatment | 3 | 2783.2750 | 927.758333 | 0.9814 ^{ns} | 2.86 | 4.38 |
| Experimental Error | 36 | 34032.7000 | 945.352778 | | | |
| Total | 39 | 36815.97500 | | | | |
| | | | | | cv | 154.457 |

Table 4. T-test and percentage decrease of the fasting blood sugar after medication of the different treatments.

| Treatment | Fasting Blood Sugar | Fasting Blood Sugar After Treatment | % Decrease | t-test | Decision |
|---|---------------------|-------------------------------------|------------|-------------|----------|
| T1 Distilled water | 88 | 155.1 | -76.25 | 4.107060101 | ** |
| T2 <i>L. babuyanica</i> Ethanolic Extract | 110.1 | 73.5 | 33.24 | 4.807473478 | ** |
| T3 Extract <i>D. falcata</i> Ethanolic | 88.6 | 67.2 | 24.15 | 2.412374907 | * |
| T4 <i>P. costalis</i> Ethanolic Extract | 100.8 | 82.8 | 17.85 | 2.747104631 | * |

Table 4 shows the percentage decrease of the blood sugar after medication. It shows that Treatment 2 (*L. babuyanica*) has the highest decrease of blood sugar with 33.24% followed by the two kulkulasi leaf decoctions T3 and T4 with 24.15% and 17.85% respectively. On the other hand, T1 (negative control), shows an increase of blood sugar with 76.25%. T-test between the baseline fasting blood sugar and the

fasting blood sugar after treatment shows that T1 and T2 are highly significant while T3 and T4 are significantly different.

Also, Table 5 shows that the analysis of variance of the fasting blood sugar after the different treatments is highly significant with F- value of 19.7208 at 1% level of significance.

Table 5. Analysis of Variance of the fasting blood sugar after medication of the different treatments.

| Source of Variation | df | Sum of Squares | Mean Square | Observed F | Tabular F 5% 1% |
|---------------------|----|----------------|--------------|------------|--------------------|
| Treatment | 3 | 66041.2750 | 22013.758333 | 19.7208** | 2.86 4.38 |
| Experimental Error | 36 | 40185.7000 | 1116.269444 | | |
| Total | 39 | 106226.97500 | | | |

cv 1501.601

Table 6. Least Significant Difference between Paired Means of the Treatments.

| Treatment | Treatment Mean | Statistical Significance | Rank |
|---|----------------|--------------------------|------|
| T1 Distilled water | -67.10 | a | 1 |
| T2 <i>L. babuyanica</i> Ethanolic Extract | 36.60 | b | 4 |
| T3 Extract <i>D. falcata</i> Ethanolic | 21.40 | b | 3 |
| T4 <i>P. costalis</i> Ethanolic Extract | 18.00 | b | 2 |

Note: Any two means having a common letter are not significantly different at 5% level;

Bold face means significantly different at 1% level to the preceding letter.

Table 6 shows further the significant differences among paired treatments. The table indicates that T2, T3 and Treatment 4 are not significantly different with one another. This means that the hypoglycemic effects of the three plants are the comparable.

The result of the bioassay of the three island endemic species of plants in Cagayan on laboratory mice in terms of its hypoglycemic effect confirms the utilization of the leaf as anti-hyperglycemic by the folkloric people.

Conclusion

Based on the findings of the study, the following conclusions were drawn. After fasting the test animals, the four treatments show a decrease in the

blood glucose from 12.76% to 38. 68%. Analysis of variance shows no significant difference among the treatments, this suggest that the decrease in sugar after fasting is comparable among treatments.

After, 24 hours of medication with the three treatments and negative control distilled water, all the treatments show decrease in blood sugar, except Treatment 1 (negative control) with no medication. Instead, the blood sugar increases. Babuyan Lunas medication shows the highest percentage decrease followed by Balingagta and Igem Dagat respectively. T-test on the difference of means of the fasting blood sugar and after medication shows that Treatment 1 and Treatment 2 are highly significant, while Treatment 3 and Treatment 4 are significant.

This implies that the decrease in blood sugar after medication is significant. Comparing the mean difference of the four treatments reveals that they are significantly different. However, further analysis of the paired treatments using LSD reveals that Treatments 2, Treatments 3 and Treatments 4 are not significantly different with one another. This implies that the hypoglycemic effects of the three endemic plant ethanolic extraction is comparable. Result confirms the folkloric used of the three endemic plants as potential anti-diabetic.

Recommendation

Based on the findings of the study, the following were recommended:

1. The three endemic plant extract can be an alternative hypoglycemic agent in the absence of commercial drug.
2. Parallel study should be conducted using other preparation of plant extracts such as the use of different solvents for hypoglycemic test.
3. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

Remarks

*Paper won 2nd Place as best Research Poster during the DOH Health Research Congress held at St. Paul University Philippines last December 21-24, 2019

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