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FORMULATION, EVALUATION, AND COMPARISON OF ANTI-DIABETIC BAGO LEAVES (*GNETUM GNEMON LINN., FAMILY: GNETACEAE*) TABLETS AGAINST COMMERCIALLY AVAILABLE METFORMIN HCL TABLET

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ABSTRACT

Gnetum gnemon L. tree grows in tropical areas and is native to countries like in the Philippines. The main purpose of this study is to find new anti-glycemic drug by formulating the tablet dosage form of Bago leaves. In statistical results, sample tablets was compared and evaluated with Metformin tabets. During the tests, weight variation (4.60%), friability (0.60), hardness (4.30), and disintegration tests (7.50) of Bago tablets got a good result. The value of each tests for sample tablets are near with the market available anti-diabetic tablet of Metformin except from its color (yellow to brownish white). Bago tablets shown promising effects that can lead to produce a good or better product than available anti-diabetic drugs. In conclusion, the Bago tablets derived from *Gnetum gnemon L.* can be a substitute drug for Metformin tablets which act as anti-glycemic drug in patients with diabetes mellitus.

KEYWORDS: Bago leaves effect in diabetes, Metformin HCl, Anti-diabetic tablet of Gnetum gnemon, Treatment for type II diabetes, Use of *Gnetum gnemon L* in diabetic patient.

INTRODUCTION

The plant of *Gnetum gnemon L.* is a sapling that can be found in the Philippines, constituting leaves are proven to have anti-diabetic properties and have similarities to angiosperm leaves. It is commonly called as "bago tree" in the Philippines. This plant grows domestically in Asia, it is practical for Filipinos to utilize the different benefits we can get out of this plant. Based on the practicality, this plant can be easily grown and not expensive to maintain. The different parts of the plant can be utilized in culinary, medical, and practical uses.

The primary objective of this study is to formulate a tablet dosage form of Bago leaves extracts and to evaluate it for pre-formulation studies and quality control test. Also, to compare the formulated sample tablets with Metformin tablets. Specifically, the proponents of the study would like to assess the following:

- The stability of the plant extracts when formulated as a tablet dosage form.
- The concentration in which the bago tablets will produce a significant anti-diabetic effect.
- The applicability of the bago tablets as an alternative for Metformin HCl tablets.
- The effectiveness of the formulated tablets compared to Metformin HCl tablets.
- The maximum toxicity of the formulated Bago leaves extract.

Recent studies determined that there are possible health benefits upon consumption of *G. gnemon L.*, various parts of the plant show positive and beneficial effects for a person. The seed extracts protect against oxidative stress inside the body, while the edible parts of the plant show antioxidant activity.

The leaf and peel extracts can decrease uric acid blood levels in hyperuricemia rats.[1] It was found out that the fruit and seeds of *G. gnemon L.* are rich in stilbene, specifically resveratrol, which is probably the cause of these plant activities.[2]

Gnetum gnemon L. has a wide variety of medicinal uses, first the plant has a high amount of zinc and can be used as a treatment for dog bites and can help in supporting proper kidney function.[3] Second the leaf sap of the plant is often used medicinally to treat eye complications.[4] Recent studies involving *Gnetum gnemon L.* shows that the leaves of the plant contain anti diabetic properties. Other studies also related to the said plant showed that the seeds and peel extract contain anti-bacterial, anti-microbial, anti-aging, antioxidant and angiogenesis – inhibitory properties.[5]

MATERIALS AND METHODS

Sample collection

Manual collection of plant samples will be conducted on Matalom, Leyte, the fresh leaf samples of *Gnetum gnemon L.* will be air-dried at room temperature. After the moisture of the leaves will be removed it will undergo extraction, ethanol extractives will be collected using an alcohol solvent. During the procurement process of the plant extract, the animal models, Male Swiss Albino Mice will be induced with type 2 diabetes using high-fat diet pellets for 30 days and low-dose (30mg/kg) streptozotocin through intraperitoneal injection. Glucometer apparatus will be used in measuring the blood collected from the tail vein.

Extraction methods

Fresh leaves of Bago leaves were collected from Matalom, Leyte and will be used in the present study. It will be thoroughly washed with tap water and dry for 20 to 30 days and powderized. The powdered plant materials are extracted by using the maceration process and used the Rotary Evaporator after.

Alcohol Extract (Ethanol)

The shade dried coarsely powdered plant leaves of *Gnetum gnemon L.*(mg) has been extracted with alcohol (75-78°C), until the extraction is completed. After the completion of the extraction, the solvent was removed by distillation. The residue has been then stored in a desiccator.[6]

Angle of Repose

This was determined by using the funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap or head of the blend. The drug excipient blend was allowed to flow to the tunnel freely on the surface.[7]

Loose Bulk Density

It was determined by pouring a weighted quantity of blend into a graduated cylinder and measuring the volume and weight.[7]

Tapped Bulk Density

It was determined by placing a graduated cylinder, containing a known mass of drug excipient blend. The cylinder was allowed to fall under its own weight onto a hard surface from a height of 10 cm at two second intervals. The tapping was continued until no further change in volume was noted.[7]

Compressibility Index

The compressibility index of the blends was determined by Carr's compressibility index.[7]

Hausner Ratio

It is the measurement of frictional resistance of the drug. The ideal range should be 1.2 - 1.5. It is determined using the following formula.[7]

Weight Variation Test

For weight variation, 20 tablets average weight was determined N individually each tablet was examined individually each tablet weight was examined. In case each deviation from the average weight was calculated and expressed as percentage.[7]

Friability

Ten tablets of each formulation were accurately weighed and placed in a drum of a friabilator and rotated at 25 rmp for a period of 4 min. The tablets were then brushed and reweighted. The percentage loss in weight was calculated and served as a measure of friability.[7]

Hardness

Ten tablets of each formulation were tested for hardness using a Monsanto tablet hardness tester, USA. The mean hardness in kilograms was then determined.[7]

Disintegration Time

The disintegration time for each six tablets of each formulation was determined using a USP disintegration tester (Pharma Test, Type PTZ2, Germany)in accordance with standard testing procedures.[7]

Selection of Test Animal

The Sprague-Dawley rat is anatomically suitable for the study due to its size and ease in handling. It is also preferable over rabbits due to its smaller size and weight. Previous studies have also utilized Sprague-Dawley rats for pharmacological assay. The testing's duration of time frame will take approximately 21 days. The source of the animals are from University of the Philippines -Diliman.

RESULTS AND DISCUSSION

This shows the experiment's results as proposed in the methodology. The tables and figures are used to support the interpretation of the results.

1. Are the plant extracts stable when formulated as a tablet dosage form?

Based on the Test for In Process Quality Control, the tablet passed all the test performed. For Metformin: it has a weight variation of 4.83%, friability of 0.58, Hardness of 3.50 kg/cm2 and disintegration of 6.00 which all passed within the given range. For the sample Bago: it has a weight variation of 4.60%, friability of 0.60, Hardness of 4.30kg/cm2 and disintegration of 7.50 which also all passed within the given range.

2. Does the Bago leaves extract have a significant anti-diabetic effect?

The result shows that Bago leaves (*Gnetum gnemon L.*) extract has a significant anti-diabetic effect which can be used as an alternative for natural therapy in cases of Diabetes. The succeeding tables below will show and explain the significant anti-diabetic effect of the formulated Bago leave tablets.

Table 1
Group A - Control Group.

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	102	111	103
RAT 2	82	88	99
RAT 3	76	84	79
RAT 4	84	70	79
MEAN	86	88.25	90
SD	11.19523708	17.01714821	12.80624847

Table 1 serves as the control group and received no treatment or modification on diet that is why the post-induction with an average of 88.25 was lower than the other groups and no change occurred on the post-treatment with an average of 90.

Table 2
Group B - Negative Control.

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	70	209	130
RAT 2	104	210	134
RAT 3	89	228	168
RAT 4	106	244	145
MEAN	92.25	222.75	144.25
SD	16.66083231	16.64081328	17.052627939

Table 2 serves as the negative control and received a high-fat diet and high-glucose diet that explains the increase on its glucose level with an average of 222.75 and there was no treatment administered.

Table 3
Group C - Metformin (500 Mg).

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	77	200	117
RAT 2	90	223	111
RAT 3	95	225	107
RAT 4	86	205	109
MEAN	87	213.25	111
SD	7.615773106	12.6062154	4.320493799

Table 3 serves as a positive control group and receives 500 mg of metformin for the treatment of glucose levels of high-fat and high-glucose diet induced on the negative group. This serves as the standard for the comparison to Bago tablets 500 mg. Upon analyzing the post-treatment, it shows that Metformin 500 mg was able to decrease the blood glucose levels from 213.25 to 111.

Table 4
Group D - Bago (500 Mg).

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	86	206	103
RAT 2	85	220	119
RAT 3	87	217	109
RAT 4	92	246	105
MEAN	87.5	222.25	109
SD	3.109126351	16.93861466	7.118052168

Table 4 serves as a treatment group and receives 500 mg of bago leaves. Upon analyzing the post-treatment, it shows that Bago 500 mg was able to decrease the blood glucose levels from 222.25 to 109. Comparing this result against Metformin 500 mg, it shows that Bago 500 mg has greater glucose lower effect than the standard Metformin 500 mg.

Table 5
Group E - Metformin (850 Mg).

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	91	218	109
RAT 2	56	232	100
RAT 3	75	249	110
RAT 4	79	201	96
MEAN	75.25	225	103.75
SD	14.52297031	20.41241452	6.849574196

Table 5 serves as a positive control group and receives 850 mg of metformin for the treatment of glucose levels of high-fat and high-glucose diet induced on the negative group. This serves as the standard for the comparison to Bago tablets 850 mg. Upon analyzing the post-treatment, it shows that Metformin 850 mg was able to decrease the blood glucose levels from 225 to 103.75.

Table 6
Group F - Bago (850 Mg).

Rat	Baseline	Post-Induction	Post - Treatment
RAT 1	99	231	93
RAT 2	59	206	110
RAT 3	104	249	113
RAT 4	97	226	95

MEAN	89.75	228	102.75
SD	20.71030339	17.68238295	10.21028893

Table 6 serves as a treatment group and receives 850 mg of bago leaves. Upon analyzing the post-treatment, it shows that Bago 850 mg was able to decrease the blood glucose levels from 228 to 102. Comparing this results against Metformin 850 mg, it shows that Bago 850 mg has greater glucose lower effect than the standard Metformin 850 mg.

1. **Is the bago leaves extracts suitable as an alternative for Metformin HCl tablets for type 2 diabetes mellitus therapy?**

Yes, because based on the results of Bago at 500 mg and 850 mg it shows the potential to reduce sugar level which makes Bago leaves tablet suitable as an alternative for Metformin HCl tablets for type 2 diabetes mellitus therapy.

Statistical results

Table 7

Average Glucose Level Per Treatment.

Treatment	Baseline	Post-Induction	Post-Treatment
Control Group	86.00	88.25	90.00
Negative Control	92.25	222.75	144.25
Metformin 500 mg	87.00	213.25	111.00
Bago 500 mg	87.50	222.25	109.00
Metformin 850 mg	75.25	225.00	103.75
Bago 850 mg	89.75	228.00	102.75

Test For Significant Difference (Between Groups)

Table below shows the test for significant difference (between groups) in Post-Treatment. This would mean that each group is compared with each other in terms of the glucose count in post-treatment.

Table 7.1. ***Significant difference between groups.***

Compared Treatment		p-value	Significance
Control Group	Negative Control	.000	Significant
	Metformin 500 mg	.012	Significant
	Bago 500 mg	.021	Significant
	Metformin 850 mg	.083	Not Significant
	Bago 850 mg	.106	Not Significant

For the control group, the computed p-value when compared to negative control, Metformin 500 mg, and Bago 500 mg is less than 0.5 alpha level. This would mean that there is a significant difference. Hence, the glucose count of the Control group in post treatment is significantly lower than the glucose count of Negative Control, Metformin 500 mg, and Bago 500 mg. However, the computed p-value when compared to Metformin 850 mg and Bago 850 mg is greater than .05 alpha level. This would mean that there is no significant difference in the glucose count when Control group is compared to Metformin 850 mg and Bago 850 mg.

Table 7.2. ***Significant difference between groups.***

Compared Treatment		p-value	Significance
Negative Control	Metformin 500 mg	.000	Significant
	Bago 500 mg	.000	Significant
	Metformin 850	.000	Significant
	Bago 850 mg	.000	Significant

For Negative control, the computed p-value when compared to Metformin 500 mg, Bago 500 mg, Metformin 850 mg, and Bago 850 mg is less than .05 alpha level. This would mean that there is a significant difference. Hence, the glucose count of negative control in post treatment is significantly higher than the glucose count of Metformin 500 mg, Bago 500 mg, Metformin 850 mg, and Bago 850 mg.

Table 7.3. ***Significant difference between groups.***

Compared Treatment		p-value	Significance
Metformin 500 mg	Bago 500 mg	.793	Not Significant
	Metformin 850 mg	.347	Not Significant
	Bago 850 mg	.286	Not Significant

For Metformin 500 mg, the computed p-value when compared to Bago 500 mg, Metformin 850 mg, and Bago 850 mg is greater than .05 alpha level. This would mean that there is no significant difference. Hence, Metformin 500 mg has the same effect in lowering glucose with Bago 500 mg, and Bago 850 mg.

Table 7.4. ***Significant difference between groups.***

Compared Treatment		p-value	Significance
Bago 500 mg	Metformin 850 mg	.493	Not Significant
	Bago 850 mg	.416	Not Significant

For Bago 500 mg, the computed p-value when compared to Metformin 850 mg, and Bago 850 mg is greater than .05 alpha level. This would mean that there is no significant difference. Hence, Bago 599 mg has the same effect in lowering glucose with Metformin 850 mg, and Bago 850 mg.

Compared Treatment		p-value	Significance
Metformin 850 mg	Bago 850 mg	.895	Not Significant

For Bago 850 mg, the computed p-value when compared to Bago 850 mg is greater than .05 alpha level. This would mean that there is no significant difference. Hence, Bago 500 mg has the same effect in lowering glucose with Bago 850 mg.

Test For Significant Difference Between Post-Inducement And Post Treatment (Within Groups)

Table 8. ***Significant difference between post-inducement and post-treatment.***

Treatment	p-value	Significance
Control Group	.863	Not Significant
Negative Control	.863	Not Significant
Metformin 500 mg	.000	Significant

Bago 500 mg	.000	Significant
Metformin 850 mg	.000	Significant
Bago 850 mg	.000	Significant

Table above shows the test for significant difference between Post-Inducement and post treatment within a group. This would mean that we are checking significant increase/decrease of glucose count between post inducement and post-treatment per group.

For the control group and negative control group, the computed p-value is greater than .05 alpha level. This would mean that there is no significant difference. Hence, glucose level is not improved with control and negative control group after post treatment. For metformin 500 mg and 850 mg, the computed p-value is less than .05 alpha level. This would mean that there is a significant difference. Therefore, the glucose level is significantly lower after post treatment when Metformin 500 mg and 800 mg is used.

Test For In-Process Quality Control (Physical Parameters) Of Metformin And Bago Tablets

The formulation prepared using wet granulation method (Himaja, N., et al., 2015). Wet granulation process started from dry mixing > wet mixing > milling of the wetted mass > drying > milling of dried mass > final blending > and compressing. The end point of mixing was determined by inspection of the crumbles under moderate pressure, when it forms a clean cut. After compression of the tablets were assessed by physical parameters, results showed on table 9 and 10 below.

Table 9. ***Evaluation studies of Metformin and Bago tablets.***

	Specification	Metformin	Sample (Bago)	Remarks
Color		White	Yellow to brownish white	
Weight variation	+ - 5%	4.83%	4.60%	Passed
Friability	+ - 0.05	0.58	0.60	Passed
Hardness	2.50– 5.00 kg/cm2	3.50 kg/cm2	4.30	Passed
Disintegration	+ - 0.30	6.00	7.50	Passed

In evaluating and comparing metformin and bago tablet, weight variation, friability, hardness, and disintegration tests of bago tablets got a good result. The value of each tests for bago tablets are almost near with the market available antidiabetic tablet metformin except from its color.

Table 10. ***Preformulation of Metformin and Bago tablets.***

	Specification	Metformin	Sample (Bago)	Remarks
Hausner	1.12 – 1.18	1.15	1.20	Poor
Compressibility index	11-15%	13.03%	23.33%	Poor
Tapped bulk density		0.33	0.82	
Loose bulk density		0.26	0.71	
Angle of repose	25 – 30	27.15	29.10	Good

In comparing the preformulation tests for metformin and bago tablets, hausner, and compressibility index did not reached the specification. Tapped bulk and loose bulk density got no specification but its value for bago tablets are higher compared with metformin tablet while the angle of repose for bago tablets got good result and reached its specification the same with metformin tablets.

4. Is there a significant difference on the physical property between Bagoe leaves tablet and Metformin HCl tablet?

Even though the researchers weren't able to conduct the experiment regarding the comparison when it comes to physical property between Bago leaves and Metformin HCl, we will perform the experiments, under the CHED memo for face to face setup.

5. What is the maximum toxicity of the formulated Bago leaves extract?

The researchers we're not able to retrieve datas about the toxicity due to the COVID-19 crisis, the researchers will perform this under the CHED memo for face to face setup once the quarantine restrictions we're fully lifted. However, the previous group were able to perform acute toxicity studies using OECD (Organization for Economic Co-operation and Development) guidelines No. 423 for Acute Oral Toxicity Study. (Dancel, et al., 2019)

CONCLUSION

This study aimed to compare the formulated tablets with established anti-diabetic drugs in the market. Based on the tables in chapter 4, for Bago 500mg, the computed p-value when compared to Metformin 850 mg, and Bago 850 mg is greater than 0.05 alpha level. This would mean that there is no significant difference in their effect to the glucose level of high fat and glucose induced diet Sprague-dawley rats. Thus, Bago 500 mg has the same effect in lowering glucose with Metformin 850 mg, and Bago 850 mg. For Bago 850 mg, the computed p-value when compared to Metformin 850 mg is greater than 0.05 alpha level which means that there is no significant difference in its effect. Therefore, Bago 850 mg has the same effect in lowering glucose with Bago 500 mg. Bago tablet shows promising effects that can lead to produce a good or even better anti-diabetic drug than drugs available in the market. The researchers therefore conclude that Bago tablets derived from *Gnetum gnemon L.* can be a substitute drug for Metformin HCl tablets and perform the same effect in lowering glucose level of patients diagnosed with type 2 diabetes.

Recommendations

According to the data and results of the study, the succeeding statements are recommended for further enhancement of Anti-Diabetic property of Bago tablets derived from *Gnetum gnemon L.* and for its future improvement of the formulated product. Researchers would like to recommend the following:

1. Try to formulate Bago leaves in other dosage forms aside from tablets in you to determine which will give the best stability and effectiveness.
2. Try to examine the stability of Bago tablets to see what storage condition is the best for it.
3. Try to compare flowers of *Gnetum gnemon L.* with the leaves and identify which has higher efficacy.
4. Try to use other methods of drying and extracting in formulating tablets. This may lead you to create better products than the study produced.
5. Try to use other species of rats and see if there are any changes in the result.

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