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Comparative study of the anti-diabetic activity of insulin plant and cherry tree fresh leaf extracts in Albino Mice

Deven P. Coquilla[1*], Mary Joy P. Araneta[2], Suzette M. Arriza[3], Eileen A. Idpalina[4] Minie LBulay[5], Julie S. Berame[5]

[1]*Oro National High School, Agusan del Sur, Philippines*

[2]*Agusan del Sur National High School, Agusan del Sur, Philippines*

[3]*Mahaba Integrated School, Cabadbaran City, Philippines*

[4]*Bunawan National High School, Agusan del Sur, Philippines*

[5]*Caraga State University, Butuan City, Philippines*

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Abstract

Herbal medicine has become one of the considered alternatives in curing many diseases, including diabetes mellitus. It was found that Insulin Plant (*Costus pictus*) and Cherry Tree (*Muntingia calabura)* contain compounds that can help in treating diabetes. This study aims to investigate and compare the anti-diabetic activity of fresh leaf extracts of the Insulin plant and Cherry tree in a diabetes-induced Albino mouse (*Mus musculus*) at different concentrations. To attain the objectives, researchers utilized and prepared fresh leaf extracts of the Insulin plant and Cherry tree at different concentrations. The blood glucose level of the mice from each setup was tested every after 3 days within 15 days using a glucometer to monitor blood sugar level. After the experimentation, the results showed that as the concentration of the fresh leaf extracts both from the insulin plant and cherry tree increases, the dropping of blood glucose level also increases which indicates that administering higher concentration of the insulin plant and cherry tree fresh leaf extract increases the effectivity in lowering the blood glucose level. From the results, it can be concluded that the Insulin plant and Cherry Tree fresh leaf extract have the promising anti-diabetic property that could be of great help in the field of medicine.

* **Corresponding Author:** Deven P. Coquilla ✉ deven.coquilla@deped.gov.ph

Introduction

Diabetes mellitus is a serious chronic disease, a health problem that greatly affects the lives of individuals worldwide. According to the World Health Organization (2021), the number of cases of diabetes worldwide rose from 108 million in 1980 to 422 million in 2014. In 2019, (WHO, 2021) cited that diabetes was the ninth leading cause of death with an estimated 1.5 million deaths directly caused by diabetes. In the Philippines, diabetes is the 4th leading cause of death among Filipinos based on the data from the 2020 Philippine Health Statistics cited in a news article (Cudis, 2021), and over 6 million Filipinos are diagnosed to have diabetes, as declared by the Philippine Center for Diabetes Education Foundation (2016). Diabetes mellitus is a chronic disease that is caused by abnormally high blood glucose (blood sugar) levels as a result of the body's failure to produce enough insulin which allows the movement of sugar into the cells to be stored or be used as energy (Rahman *et al.*, 2021; Brutsaert, 2020). Diabetes is characterized by elevated glucose or sugar level in the blood (Sun *et al.*, 2020), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. It is linked with instabilities in the metabolism of carbohydrates, fats and proteins and the deficiency of insulin hormone (American Diabetes Association, 2013). The control of insulin of the glucose level is essential to decrease the occurrence and severity of complications diabetes would bring to the health of an individual (Dhatariya *et al.*, 2020) (Pasquel *et al.,* 2021). Despite the development of the medicine that provided essential milestones in preventing the severity of the complications as a result of diabetes, anti-diabetic medicine has limitations and side effects to the body such as hypoglycemia, weight gain, gastrointestinal problem, liver toxicity, etc., aside from the mentioned, it is costly, need to be taken throughout the lifetime, and are not easy to access in the market (Aruna *et al.,* 2014).

In this regard, there is a growing interest in herbal medicines for the treatment of diabetes mellitus. Herbal medicines are a promising choice over synthetic drugs since it is easier to access, easier to prepare and it comes with fewer or no complications and side effects (Ekor, 2014). In the Ayurvedic system of medicine, diabetes is traditionally treated by chewing the plant leaves for a period of one month to get a controlled blood glucose level (Hedge *et al.,* 2019). In the Philippines, getting fresh extracts from different parts of a medicinal plant and drinking them is evident as a substitute or alternative treatment for different diseases such as cough, stomachache, diabetes and etc. In some studies, a native plant in the Philippines called Banaba (*Lagerstroemia speciose*) is used to cure diabetes. Based on the result using Banaba (*Lagerstroemia speciose*) fresh extract for 2 weeks lowers blood sugar by 10% in people with type 2 diabetes (WebMD, 2020). This Banaba plant is native to the Philippines and Filipinos use its leaves extracts for medicine. With thorough research on the pharmacological properties of the discovered medicinal plants, it led to the discovery of medicinal plants having efficacy in treating diabetes mellitus through using its extracts. Recently, extracts of Insulin Plant *(Costus Pictus)* and Cherry Tree *(Muntingia calabura)* have been reported to show anti-diabetic properties (Selvakumarasamy *et al.*, 2021) (Solikhaht., 2021).

The Insulin plant (*Costus pictus)*, a member of the family Zingiberaceae, was discovered to be a medicinal plant known for its pharmacological properties. It is known to have anti-diabetic properties, anti-microbial properties, anticancer properties, antioxidant activities, anti-fertility, anti-helminthic, anti-inflammatory and diuretic effects (Devi, 2019). The leaves of *C. pictus* are medicinally essential in treating hyperglycemia; the ethanolic and methanolic extracts, when induced in alloxan-induced rats, showed a significant reduction in the blood sugar level (Remya *et al.,* 2013). In a study conducted by Aruna *et al.* 2014, entitled "Comparative Anti-diabetic Effect of Methanolic Extract of Insulin Plant *(Costus pictus)* Leaves and its Silver Nanoparticles", it was found that the leaves of *Costus pictus D. Don* can be a good source for the synthesis of silver nanoparticle which showed potential antioxidant and anti-diabetic

activity. (Aruna *et al.,* 2014) also cited that the anti-diabetic activity of *Costus pictus D. Don* might be due to the presence of pentacyclic triterpene compounds such as β- amyrin and β- L- Arabinopyranose methyl glucoside in the plant. One study discovered that Insulin Plant (*Costus pictus*) could lower blood sugar levels in mice after 21 days of treatment (Romaiyan *et al.,* 2010). Also, a phytochemical examination of the Insulin Plant (*Costus pictus*) found the presence of phenols and flavonoids, which are known components of potential diabetic treatments (Gupta *et al.,* 2013).

Another plant that was found to be having potential anti-diabetic activity is the Cherry tree (*Muntingia calabura*). *M. calabura* is known in the Philippines as "Aratilis". Same with other medicinal plants, *M. calabura* was found to be having pharmacological activities such as; antinociceptive, cardioprotective, antipyretic, antiplatelet aggregation, antioxidant, anti-inflammation, anti-diabetic, anti-ulcer, and antibacterial (N.D. Mahmood *et al.*, 2014). It was found that *M. calabura* has anti-diabetic activities with mechanisms to lower blood glucose levels, have the ability to regenerate pancreatic β cells, and increase insulin sensitivity which helped in lowering the blood glucose level in the body (Aligita *et al.,* 2018) (Herlina *et al. 2018).* In alloxan-induced diabetic rats, the leaf extract of *Muntingia calabura L.* significantly lowered the blood glucose levels to an extent comparable to that produced by the standard anti-diabetic drug and in a glucose-loaded rat, the extract increases the glucose tolerance of the rat which suggest that the methanolic extract of *Muntingia calabura* leaves possess significant anti-diabetic activity (M. Sridhar *et al.,* 2011). The leaves of *Muntingia calabura* contain saponins and flavonoids, specifically quercetin which can reduce blood sugar levels which help facilitate anti-diabetic activity (Shafira *et al.*, 2021). Another study also shows that the *M. calabura* leaves, when extracted with 50% ethanol and dried using FD Method, can extract anti-diabetic metabolites which can be a potential for the development of naturally-derived herbal medicinal components that not only help to inhibit diabetes-related complications but also impedes toxic side effects of synthetic anti-diabetic drugs (Zolkeflee *et al.*, 2022). According to one study, Cherry tree leaves are the most effective part of the cherry tree in lowering blood glucose levels (Omamos, 2018). The *M. calabura* leaves contain an important component of anti-diabetes properties such as anthocyanin, flavonoid and polyphenol and have the potential to cure type 2 diabetes (Layson, 2019).

With this background, the study has attempted to investigate and compare the anti-diabetic activity of fresh leaf extracts of Insulin plant *(Costus pictus)* and Cherry tree (*Muntingia calabura*) in a diabetes-induced Albino mouse (*Mus musculus*). The aim of the study is to meet the following objectives: to determine the anti-diabetic activity of Insulin Plant *(Costus pictus)* fresh leaf extract in Albino Mice *(Mus musculus)* at different concentrations; to determine the anti-diabetic activity of Cherry tree *(Muntingia calabura)* fresh leaf extract in Albino Mice *(Mus musculus)* at different concentrations, and to compare and determine the significant difference on the anti-diabetic activity of Insulin Plant *(Costus pictus)* and Cherry tree *(Muntingia calabura)* fresh leaf extracts in Albino Mice *(Mus musculus)* at different concentrations.

Materials and methods

Research design

This study will use the true experimental design in which it will use a control group and experimental groups that will help to determine and compare the anti-diabetic activity of insulin plant (*Costus pictus*) and cherry tree (*Muntingia calabura*) fresh leaf extracts in diabetes-induced albino mice (*Mus musculus*).

Research locale and sample

The study was conducted in Agusan del Sur, Philippines, since each experimental setup needed daily monitoring. The researchers collected the plant leaves, the Insulin plant (*Costus pictus*) and the Cherry tree (*Muntingia calabura*). The researchers used Albino mice (*Mus musculus*) weighing 20g-30g

as the test subject for the anti-diabetic activity of the Insulin plant's (*Costus pictus*) and Cherry tree's (*Muntingia calabura*) fresh leaf extracts.

Ethical considerations

As the experimental study deals with the Albino Mice (*Mus musculus*), the researchers guarantee that considerations are undertaken making sure that there are no laws or ethics that were being violated in this study. The researchers made sure to adhere to the 3Rs: Replacement, Reduction and Refinement, formulated by William Russell and Rex Burch (Hubrecht *et al.,* 2019). Thus, the researchers opted to use albino mice over humans to examine the anti-diabetic activity of plant extracts; use the minimum number of mice; and take appropriate measures to mitigate any pain, suffering, or distress to the selected healthy albino mice, making sure that they were taken good care before, during, and after the experimental study. The researchers also guarantee that there were no other animals or humans harmed except for the experimental albino mice used in this study.

Instrumentation and materials

There were setups that were prepared to cater unbiased results from this study and the following materials and variables were used along the way.

Extraction of fresh leaf extract and preparation of concentrations:	
Leaves of Insulin Plant (*Costus pictus*)	Mortar and pestle
Leaves of Cherry Tree (*Muntingia calabura*)	Distilled water
100ml beaker	Cheesecloth
Stirring rod	Earthenware container
Preparation of setups and experimental procedure:	
Fresh leaf extract concentrations	Cage for each setup
Albino Mice (Mus musculus)	7 Bottles/ water dispensers
Glucose foods	Glucometer
Notebook	Ballpen

Data gathering procedure

Collection of plant samples

The researchers collected the Insulin plant (*Costus pictus*) and Cherry tree (*Muntingia calabura*) leaves. The researchers opted to make use of the leaves since Filipinos are fond of using the leaves alone to allow the stem to grow back a new set of leaves. The leaves will be washed thoroughly as preparation for fresh leaf extraction.

Extraction of plant fresh leaf extracts and preparation of concentrations

In order to extract the medicinally active portions of plant or animal tissues, the researchers used the procedure below which is based on the study "Effect of the insulin plant (*Costus igneus*) leaves on dexamethasone-induced hyperglycemia" (Shetty *et al.*, 2010).

Wash the collected leaves of Insulin plant *(Costus pictus)* and Cherry tree *(Muntingia calabura),* cut it into small strips and place it in an earthenware container separately. Using mortar and pestle, pound the prepared leaves of Insulin plant (*Costus pictus*) and Cherry tree (*Muntingia calabura*) separately. Pound continuously till it extracts the leaf juice. After pounding the leaves, filter separately the fresh leaf extracts using a cheesecloth and then prepare it for the different concentrations. Prepare the 3 different concentrations in each of the fresh leaf extracts of the Insulin plant *(Costus pictus*) and Cherry tree *(Muntingia calabura*) leaves: for 1L solution with 30% concentration of leaf extract, use 300ml of the leaf extract and 700ml of distilled water; for 1L solution with 60% concentration of leaf extract, use 600ml of the leaf extract and 400ml of distilled water; and for 1L solution with 90% concentration of leaf extract, use 900ml of the leaf extract and 100ml of distilled water.

These concentrations will be used in their respective setups as drinking water of the albino mice *(Mus musculus*) for oral intake of the decocted leaves extract.

Experimental procedure

Albino mice (*Mus musculus*) will be used in this experiment because these animals share the same cells, tissues and organs as humans; they offer the

best indicator of how humans will react to a new diabetes drug or medical treatment (BioChemed, 2019). The experimental procedure that will be used in this experiment is taken from the article by Tafesse *et al.*, 2017 entitled Anti-diabetic Activity and Phytochemical Screening of Extracts of the Leaves of Ajuga remota Benth on Alloxan-Induced Diabetic Mice; the study of Hidayaturrahmah *et al.*, in 2020 entitled Blood glucose level of white rats (*Rattus norvegicus*) after giving catfish biscuit (*Pangasius hypothalmus*); and the article of Benedé-Ubieto *et al.* (2019) entitled Guidelines and Considerations for Metabolic Tolerance Tests.

The researchers selected and used Albino mice (*Mus musculus*) weighing 20g to 30g for the anti-diabetic activity experiment. The researchers divided the 21 albino mice into 7 setups, 3 each setup. The albino mice were fed with high-glucose foods for seven days to raise their blood glucose levels. The albino mice underwent fasting for about 12 hours to measure their fasting blood glucose level using a glucometer before and after feeding with the high-glucose foods. One (1) setup was for the control group; this setup did not receive any of the leaf extracts. The first 3 setups were given the insulin plant *(Costus pictus*) fresh leaf extracts at different concentrations, respectively; Setup A.1 will receive the 30% concentration, Setup B.1 will receive the 60% concentration, and Setup C.1 received the 90% concentration as the drinking water of the albino mice (*Mus musculus*). The other 3 setups received the cherry tree (*Muntingia calabura*) fresh leaf extracts at different concentrations, respectively; Setup A.2 will receive the 30% concentration, Setup B.2 will receive the 60% concentration, and Setup C.2 will receive the 90% concentration as the drinking water of the albino mice (*Mus musculus*). In every three days, the blood sample of the albino mice in each setup was collected by pricking its tail part. Still, the albino mice undergo fasting for about 12 hours before taking the blood sample. The fasting blood sugar level of the albino mice was measured and monitored. The monitoring of the blood sugar level covered 15 days, and happened on the 1st day as the initial day, 3rd day, 6th day, 9th day, 12th day and lastly, 15th day after drinking the prepared concentrations. Though the control group did not receive the extract concentrations, the blood glucose levels of the Albino mice in the setup were also monitored, same with the other setups using glucometer apparatus. The data gathered from the monitoring of the blood sugar level of the albino mice were recorded for the data analysis.

Statistical treatment of data

Based on the needs of the study, the researcher used statistical tools to analyze and interpret the data and to test the null hypotheses. These include weighted mean and ANOVA. Weighted Mean will be used to determine the final average to signify the relative importance of each data and to smooth out data, thus improving accuracy. ANOVA will be used employed to determine the significant difference between the two groups. The level of significance that will be employed in this study is 0.05 or 95% level of confidence to determine the significance of the findings.

Result and discussion

Insulin plant (*Costus pictus*) was reported to have many important and beneficial aspects which include anti-diabetic, anti-microbial, anti-cancer, antioxidant, anti-fertility, anti-helminthic, diuretic, and anti-inflammatory properties (Devi, 2019). Muthukumar *et al.* (2019) stated that it is evident that the leaves of the insulin plant contain the maximum amount of phytocomponents. According to Aruna *et al.* (2014), preliminary phytochemical screening on insulin plant leaves showed the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, sterols and volatile oil. Extracts of insulin plant leaves have shown a maximum number and concentration of secondary metabolites (Shiny *et al.*, 2013).

In the study of Selvakumarasamy *et al.* (2021), *C. pictus* leaves showed high concentrations of flavonoids, namely isoquercetin, astragalin, kaempferol and quercetin. Quercetin is the main compound that regenerates the pancreatic β-cells and stimulated the release of insulin, hence promoting the

anti-diabetic properties. Daucosterol (*β*-sitosterol-3-O-*β*-D-glucoside) isolated from the leaves of *Costus pictus* displayed anti-hyperglycemic properties. According to a review of anti-diabetic phytochemicals of medicinal plants authored by Safaet *et al.* in 2022, β- L- Arabinopyranose methyl glycoside was reported to be responsible for the anti-diabetic property of *C. pictus*. *Costus pictus* extracts could stimulate insulin secretion from β-cells of islets of Langerhans (Al-Romaiyan *et al.,* 2010).

Table 1. Anti-diabetic Activity of Insulin Plant (*Costus pictus)* Fresh Leaf Extract in Albino Mice (*Mus musculus)* at Different Concentrations.

Concentration	Initial	Average Blood Glucose Level After Treatment	Decrease of Blood Glucose Level after Treatment (%)
30%	122.00	115.00	5.74
60%	122.67	106.67	13.04
90%	144.00	115.33	19.91

*Normal Blood Glucose Level is at 80 mg/dl to 100 mg/dl.

The researchers investigated the anti-diabetic activity of the insulin plant (*Costus pictus*) using its fresh leaf extract in albino mice (*Mus musculus*) at 30%, 60% and 90% concentrations.

The blood sample of the test subject was monitored every three days. There were five monitoring for 15 days to thoroughly check how it affects the blood glucose level of the albino mice.The graph in Figure 2 shows that at 30% concentration, the three albino mice exhibited changes in their blood glucose level.

It has almost the same effect on the three albino mice in this setup. From the initial day to the last day of monitoring, there is a gradual decrease in their blood glucose level. However, the data shows that the anti-diabetic activity of the insulin plant at 30% concentration has a very minimal effect.

Table 2. Test of Significant Difference in the anti-diabetic activity of Insulin Plant (*Costus pictus*) in Albino Mice (*Mus musculus*) at Different Concentrations.

Sources of Variation	Test Statistic	P-value	Conclusion
Different concentrations of Insulin Plant fresh leaf extract	26.438	0.001	Significant
**Significant at 0.05*			

In Figure 3, the graph shows that the three albino mice in this setup also exhibited changes in their blood glucose level. The two albino mice represented in blue and gray lines showed almost the same result in all the monitoring of their blood glucose level compared to the albino mice represented in the red line. All the albino mice that were given 60% concentration of the insulin plant (*Costus pictus*) showed more decrease in their blood glucose level compared to the set of albino mice that had taken only 30% concentration of insulin plant.

Table 3. Anti-diabetic Activity of Cherry Tree (*Muntingia calabura*) Fresh Leaf Extract in Albino Mice (*Mus musculus)* at Different Concentrations.

Concentration	Initial	Average Blood Sugar Level After Treatment	Decrease of Blood Glucose Level after Treatment (%)
30%	137.67	130.33	5.33
60%	119.00	107.67	9.52
90%	128.00	109.00	14.84

**Normal Blood Glucose Level is at 80 mg/dl to 100 mg/dl.*

Figure 4 shows the trend of anti-diabetic activity of insulin plant fresh leaf extracts at 90% concentration. All the albino mice in this setup show almost the same result. But, among the three concentrations, this setup shows the highest decrease in the blood glucose level of the albino mice.

Table 4. Test of Significant Difference on the anti-diabetic activity of Cherry Tree (*Muntingia calabura*) in Albino Mice (*Mus musclus*) at Different Concentrations.

Sources of Variation	Test Statistic	P-value	Conclusion
Different concentrations of Cherry Tree fresh leaf extract	86.273	0.000	Significant
**Significant at 0.05*			

Figure 5, Figure 6, and Figure 7 show the trend of the anti-diabetic activity of the fresh leaf extracts of the insulin plant (*Costus pictus*) which indicates that the insulin plant fresh leaf extract should be taken regularly at high concentrations to ensure its effectiveness in reducing blood glucose level.

The fresh leaf extract of the insulin plant (*Costus pictus*) was prepared in three different concentrations, 30%, 60% and 90%. Its anti-diabetic activity results, as shown in Table 1, present that there is a dose-dependent effect of insulin plant fresh leaf extract on the blood glucose level of albino mice (*Mus musculus*). The 90% concentration has the highest anti-diabetic activity that shows a 19.91% decrease in the albino mice' (*Mus musculus*) blood glucose level after the treatment compared to the 30% and 60% concentrations with the anti-diabetic activity of only 5.74% and 13.04%, respectively.

This result was supported by the graph in Figure 8 which shows that as the concentration of insulin plant fresh leaf extract increases, its anti-diabetic activity also increases. This indicates that the consumption of insulin plants at higher concentrations has a better effect on reducing blood glucose levels (Chowdary *et al.*, 2020). According to Mathew and Varghese (2019), fresh leaf extracts of insulin plants should be taken daily for 30 days to effectively bring the blood glucose levels towards normal.

Table 5. Test of Significant Difference on the Insulin Plant (*Costus pictus*) and Cherry Tree (M*untingia calabura*) Fresh Leaf Extract in Albino Mice (*Mus musculus*) at Different Concentrations.

Sources of Variation	Test Statistic	P-value	Conclusion
30% concentration	-0.316	0.768	Not Significant
60% concentration	4.427	0.011	Significant
90% concentration	3.740	0.042	Significant
**Significant at 0.05*			

Table 2 shows that there is a significant difference in the anti-diabetic activity of the insulin plant (*Costus pictus*) with a p-value of 0.001 which shows the effectiveness of the fresh leaf extract of the insulin plant in lowering the blood glucose level in albino mice (*Mus musculus*) is dependent to its concentration.

According to Naik *et al.* (2022), a high dose of aqueous *C. pictus* and methanolic *C.pictus* with the same amount of metformin administered to alloxan-induced rats showed promising results in reducing blood glucose levels. In addition, in the study of Hajam *et al.* (2022), it was also observed that there is a significantly higher recovery rate in a diabetic-induced rat treated with 300 mg/kg dose of insulin plant extract compared to diabetic-induced rats treated with 100 and 200 mg/kg insulin plant extract. The displayed significant difference in the anti-diabetic activity of insulin plant fresh leaf extract at different concentrations in which the higher the concentration is, the greater it reduces the blood

glucose level. It supports the idea why ingesting the leaves of *C. pictus* is advised by medicine practitioners in treating diabetes as a review of the anti-diabetic phytochemical from medicinal plants reveals that the ingestion of the leaves of *C. pictus* by diabetic patients showed a statistically significant reduction in their fasting and postprandial blood glucose levels, as per a cross-sectional clinical study (Shetty *et al.*, 2010) (Safaet *et al.,* 2022).

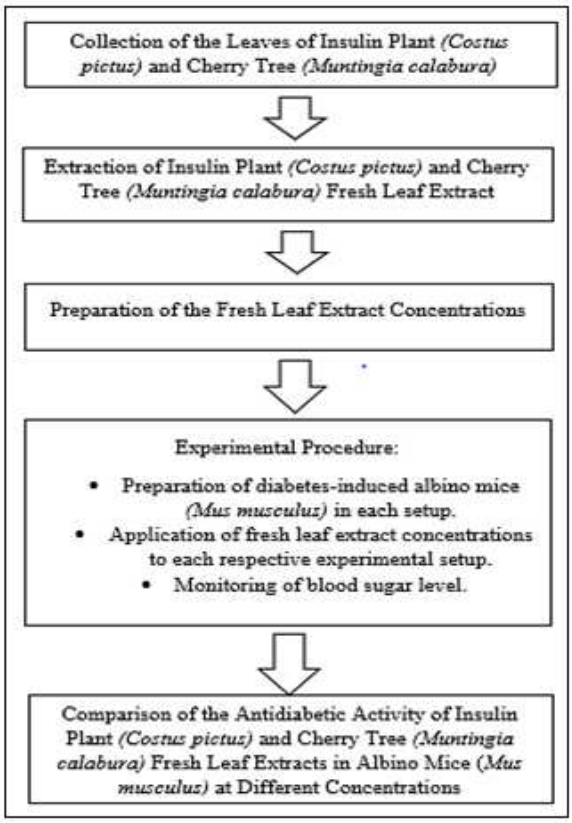

Fig. 1. Flow Chart of the Study.

Therefore, the effectiveness of the insulin plant increases as the concentration increases. Insulin plants should be regularly taken daily at high concentrations to bring the blood glucose level completely under control (Mathew *et al.*, 2019).

Anti-diabetic activity of Cherry Tree (Muntingia calabura) fresh leaf extract in Albino Mice (Mus musculus) at different concentrations?

Cherry tree (*Muntingia calabura*). *M. calabura* is known in the Philippines as "Aratilis" which exhibits promising pharmacological activities such as; antinociceptive, cardioprotective, antipyretic, antiplatelet aggregation, antioxidant, anti-inflammation, anti-diabetic, anti-ulcer, and antibacterial (N.D. Mahmood *et al.,* 2014). *Mutingia calabura,* specifically the leaves, is used as herbal medicine to cure diabetes and exhibits potential anti-diabetic activity that helps in lowering blood glucose levels (Solikha *et al.*, 2021) (Omamos, 2018). According to Andalia *et al.* in 2020, *Mutingia calabura* is known to be effective in lowering blood glucose levels because it has a component of anti-diabetic properties like anthocyanin, flavonoid, ascorbic acid, fiber, niacin and β-carotene.

It was found that *M. calabura* has anti-diabetic activities with mechanisms to lower blood glucose levels, have the ability to regenerate pancreatic β cells, and increase insulin sensitivity which helped in lowering the blood glucose level in the body (Aligita *et al.*, 2018) (Herlina *et al. 2018).*

The researchers investigated the anti-diabetic activity of the cherry tree (*Muntingia calabura*) using its fresh leaf extract in albino mice (*Mus musculus*) at 30%, 60% and 90% concentrations. The blood sample of the test subject was monitored every three days. There were five monitoring for 15 days to thoroughly check how it affects the blood glucose level of the albino mice.

Figure 6 depicts the trend of anti-diabetic activity of a 30% concentration of Cherry tree fresh leaf extract. The blood glucose level drops at the start of the 3rd day until the end of the 15th day of treating the induced albino mice with the Cherry tree fresh leaf extract. The 30% concentration of Cherry tree fresh leaf extract can effectively drop blood glucose levels, as shown on the graph. From the average initial blood glucose level to the average blood glucose level after treatments shows a significant reduction in blood glucose level.

Figure 7 shows the trend of anti-diabetic activity of *Mutingia calabura* fresh leaf extract at 60% concentration for the duration of 15 days. From the initial blood glucose levels of the three (3) mice in setup B.2, it reduced as the days went by until the 15th

day of receiving the *M. calabura* fresh leaf extract. It demonstrates that the 60% concentration resulted in a decrease in blood glucose levels in albino mice. This also suggests that a concentration of 60% is more effective than a concentration of 30% of Cherry Tree fresh leaf extract in lowering blood glucose levels in the albino mice.

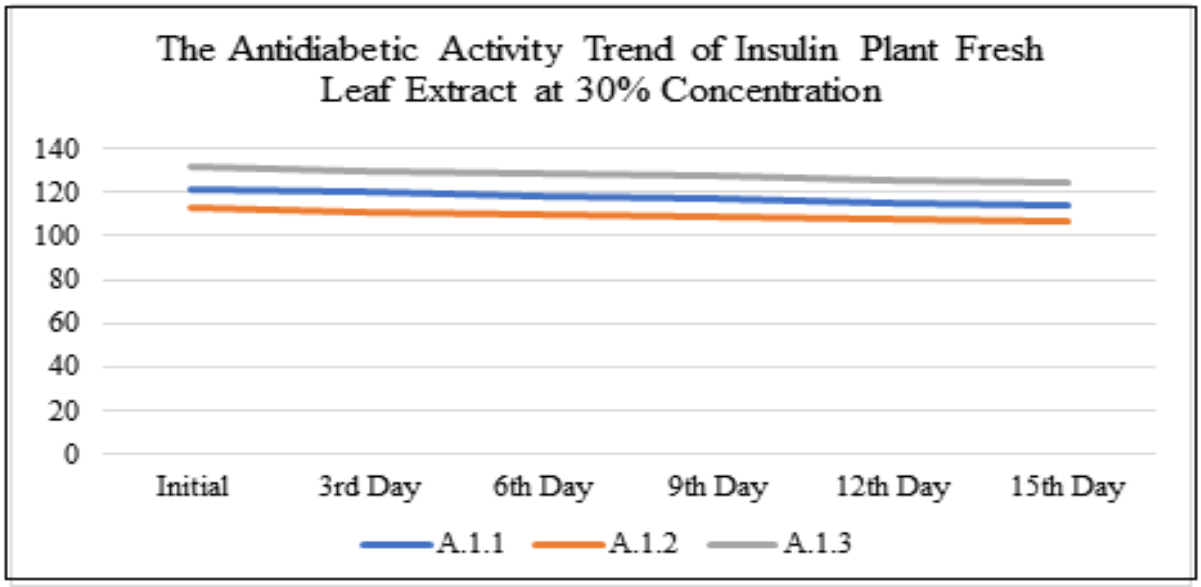

Fig. 2. Graph showing the anti-diabetic activity trend of the Insulin plant fresh leaf extract at 30% concentration.

Figure 8 shows the trend of the anti-diabetic activity of Cherry Tree fresh leaf extract at 90% concentration. It can be seen on the graph that from the initial blood glucose levels of the mice in setup B.3, it decreases until the 15th day of the treatment. This graph suggested that the Cherry tree fresh leaf extract at 90% concentration can help reduce the blood glucose level. This outcome proves that the use of Cherry Tree fresh leaf extract can effectively lower the blood glucose level despite of different concentrations. According to the findings of the study Layno (2019) stated that *Mutingia Calabura L* is a potential cure for type 2 diabetes millets.

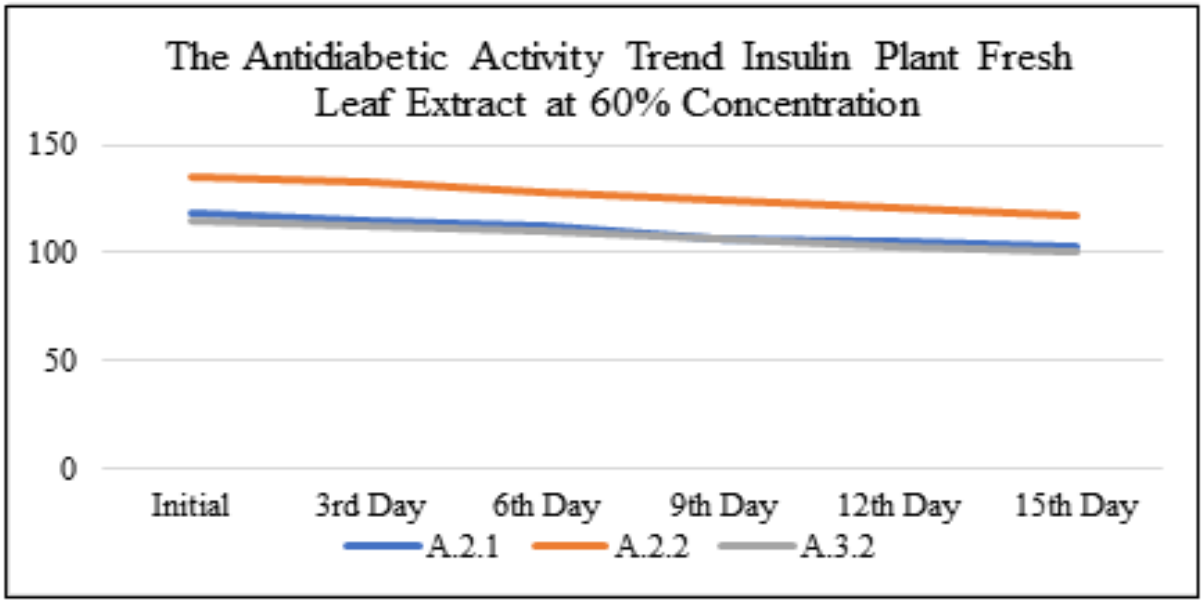

Fig. 3. Graph showing the anti-diabetic activity trend of insulin plant fresh leaf extract at 60% concentration.

Table 3 shows the different concentrations of *Muntingia calabura* and it shows how blood glucose levels drop at various concentrations. Normal blood sugar levels range from 80 to 100mg/dl. The initial blood glucose level of albino mice ranges from 110mg/dl to 165mg/dl, indicating that the albino mice's blood glucose level is above normal. After treating albino mice with Cherry Tree (Muntingia calabura) fresh leaf extracts, their blood glucose levels eventually decreased.

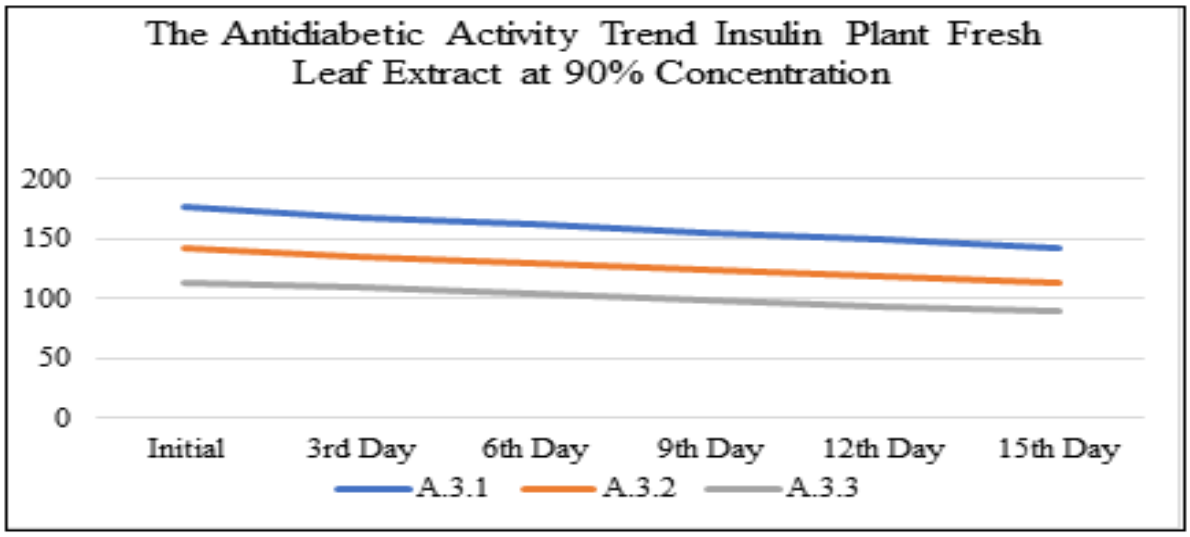

Fig. 4. Graph showing the anti-diabetic activity trend of insulin plant fresh leaf extract at 90% concentration.

The 30% concentration of Muntingia calabura exhibits anti-diabetic activity, lowering blood glucose levels by 5.33% from the average initial blood glucose level of 137.67 mg/dl, while the 60% concentration also exhibits anti-diabetic activity, lowering blood glucose levels by 9.52% from the average initial blood glucose level of 119mg/dl to average blood glucose level after treatment which is 107 mg/dl. However, the 90% concentration of Cherry Tree *(Muntingia calabura)* demonstrated the highest anti-diabetic activity, lowering blood sugar levels by 14.84%. The 90% concentration of Cherry Tree (*Muntingia calabura*) demonstrates that the higher the concentration of Cherry Tree (*Muntingia calabura*), the greater the anti-diabetic activity and the greater the effectiveness in lowering blood glucose levels.

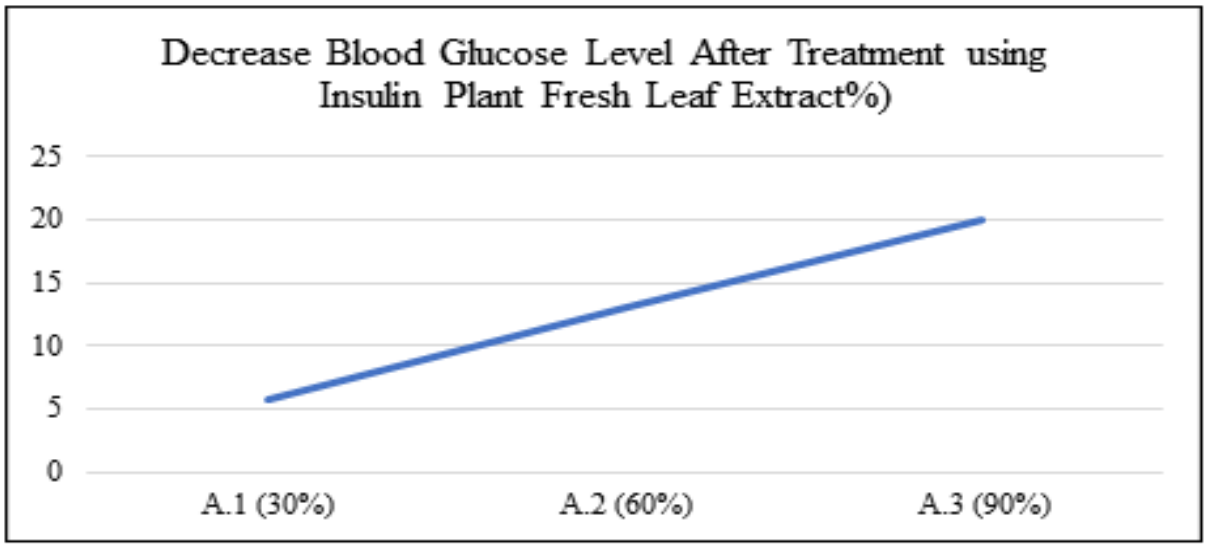

Fig. 5. Graph showing the increasing anti-diabetic activity of insulin plant fresh leaf extract as the fresh leaf extract concentration increases.

Figure 9 shows the increase of anti-diabetic activity of Cherry Tree fresh leaf extract at various concentrations. The result proves that the anti-diabetic activity of Cherry Tree fresh leaf extract in lowering blood glucose levels may vary, dependent on the amount of concentration.

These findings are indicated that the greater the amount of concentration, the more effective it is. As shown in the graph, the 90% concentration has the highest anti-diabetic activity than 30% and 60% concentration of Cherry Tree fresh leaf extract. Similar to those results of Aligata *et al.,* 2018 who found that a 400 mg/kg bw dose of *Mutingia calabura* leaves water extract had more anti-diabetic properties and blood glucose-lowering mechanisms than 100 and 200 mg/kg bw doses of Cherry Tree. Solikha *et al.*, 2021 backed up these findings, demonstrating in their study that the effect of *Mutingia calabura* leaf extract resulted in a significant reduction in blood glucose levels after 14 days of treatment with the amount of 100mg/kg and 300 mg/kg of *M. calabura* leaf extract. Solikha *et al.,* 2021 stated that the anti-diabetic effect of *M. calabura* leaf extract could normalize the weight of alloxan-induced diabetic mice.

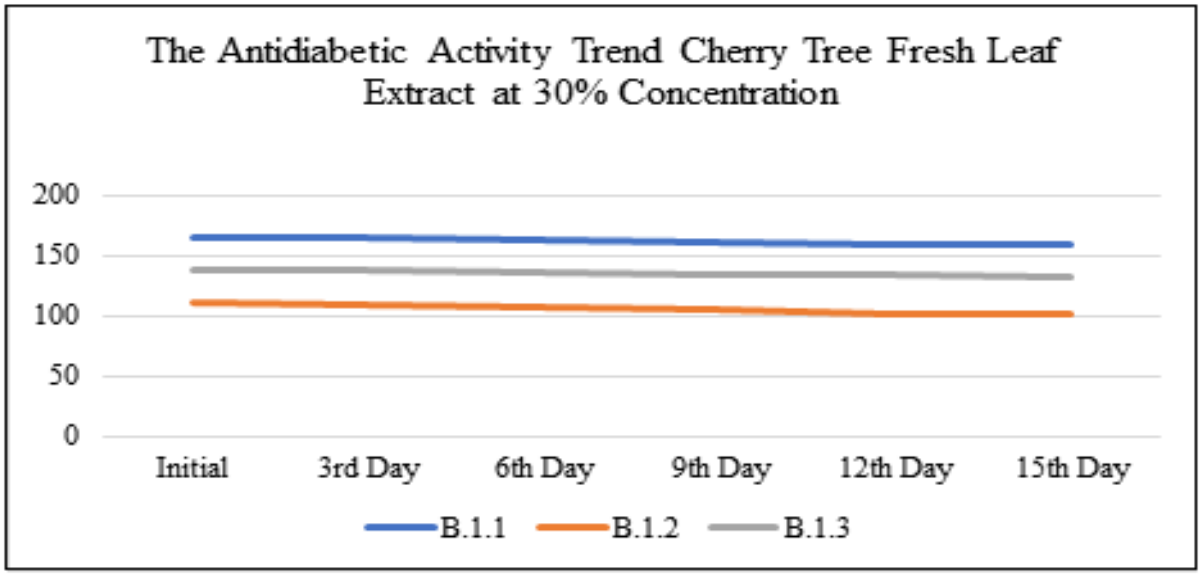

Fig. 6. Graph showing the anti-diabetic activity trend of Cherry Tree fresh leaf extract at 30% concentration.

Table 4 shows the significant difference in anti-diabetic activity of Cherry Tree fresh leaf extract at various concentrations. The result indicated that there is a significant difference in the anti-diabetic activity of Cherry Tree leaf extract in the albino mice with the value of p=0.000, indicating that the effectiveness of the fresh leaf extract of the cherry tree in lowering blood glucose levels in albino mice *(Mus musculus)* is concentration dependent. The result rejects the H_o2 which states that there is no significant difference in the anti-diabetic activity of Cherry tree *(Muntingia calabura)* fresh leaf extract in Albino Mice *(Mus musculus)* at different concentrations. The results indicate that the anti-diabetic activity of the Cherry Tree (*Muntingia calabura*) is concentration-dependent. Therefore, the higher the concentration of *Mutingia calabura* leaf extract, the lower the glucose level. A study by Andalusia *et al.* in 2021 backs up this result; the *Muntingia calabura* leaf extract was found to be effective with a P-value of 0.01; leaf extract has a very significant effect on rats' blood glucose levels. Indriawati (2020) backs up these findings with the results of their Cherry Tree leaf study, which yielded a p-value of 0.5, indicating that there is a significant difference between the five different treatments of Cherry Tree fresh leaf extract. In addition, Andalia *et al.*, 2021 concluded that *Mutingia calabura* leaf extracts 450mg/kg BW could reduce blood glucose levels in hyperglycemic rats.

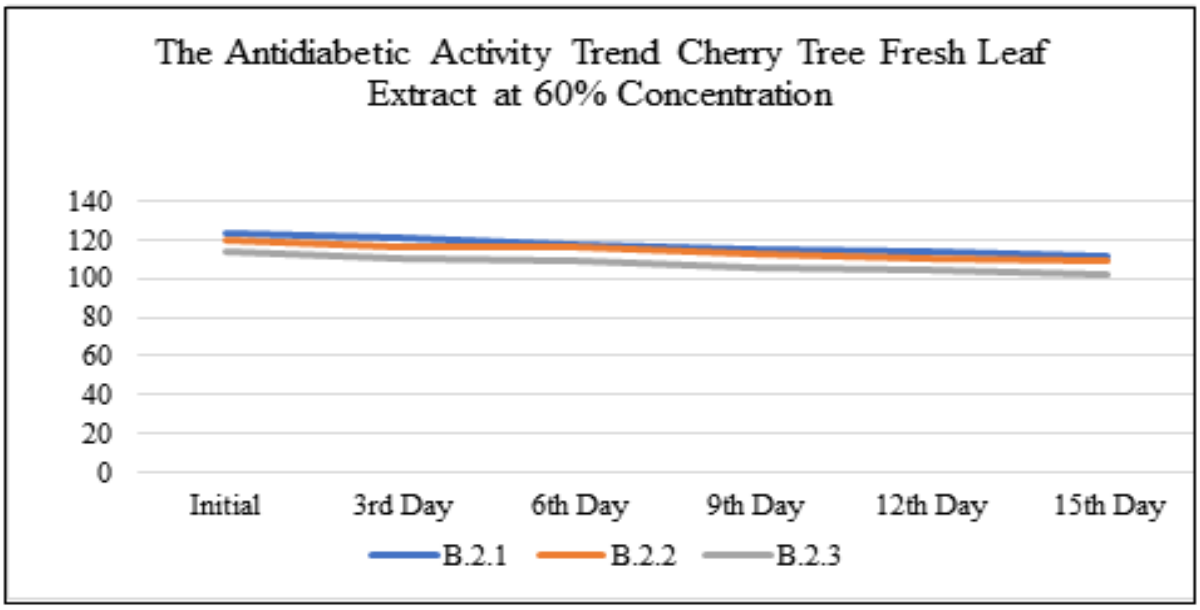

Fig. 7. Graph showing the anti-diabetic activity trend of Cherry Tree fresh leaf extract at 60% concentration.

Implication of the results only proved the effectivity of Cherry Tree leaf extract in treating diabetes is dependent on the level of concentrations. As the level of concentration increases, it also increases the level of anti-diabetic activity of the Cherry Tree (*Muntingia calabura)* fresh leaf extract in lowering the blood glucose level of the albino mice.

Significant difference on the Insulin Plant (Costus pictus) and Cherry Tree (Muntingia calabura) fresh leaf extract in Albino Mice (Mus musculus) at different concentrations

Leaf extracts of Insulin Plant *(Costus pictus)* and Cherry Tree *(Muntingia calabura)* are known to be effective herbal medicine for diabetes. Extracts of Insulin Plant *(Costus Pictus)* and Cherry Tree *(Muntingia calabura)* have been reported to show anti-diabetic properties (Selvakumarasamy *et al.*, 2021) (Solikhah t., 2021). Solution with different concentrations of leaf extracts of both herbal plants may have or may have no significant difference when taken. The table below shows if there is a significant difference in the Insulin Plant *(Costus pictus)* and Cherry Tree *(Muntingia calabura)* fresh leaf extract in Albino Mice *(Mus musculus)* at different concentrations. Table 5 shows that there is no significant difference in the anti-diabetic activity of Insulin Plant (*Mus musculus*) and Cherry Tree (M*untingia calabura*) at 30% concentration. It shows that the p-value is greater than 0.05 level significance (α=0.05) which means it failed to reject H_o. However, this table shows also that there is a significant difference in the anti-diabetic activity of Insulin Plant (*Mus musculus*) and Cherry Tree (M*untingia calabura*) at 60% concentration and 90% concentration because its *p*-value are 0.011 and 0.042, respectively are less than 0.05 level of significance which means it rejects H_o. According to Sanjay, K. in 2018, if utilized properly, high concentration insulin can offer efficient and effective glycemic control to persons requiring high doses of insulin in a safe and well-tolerated manner.

One study was conducted that used the leaf extracts of *Punica granatum to test the* anti-diabetic, hypolipidemic and antioxidant activity of hydroalcoholic extract of leaves and fruit peel of *Punica granatum* in male Wistar albino rats. The results revealed that leaves extract at a higher dose and fruit extract at a lower dose also significantly lowered blood glucose levels from 14th day onwards. Leaves extract at a lower dose also significantly lowered blood glucose levels from 21st day onwards (Salwe *et al.*, 2015). The result indicated that different concentrations of extracts play significant roles in lowering the blood glucose level of the rats.

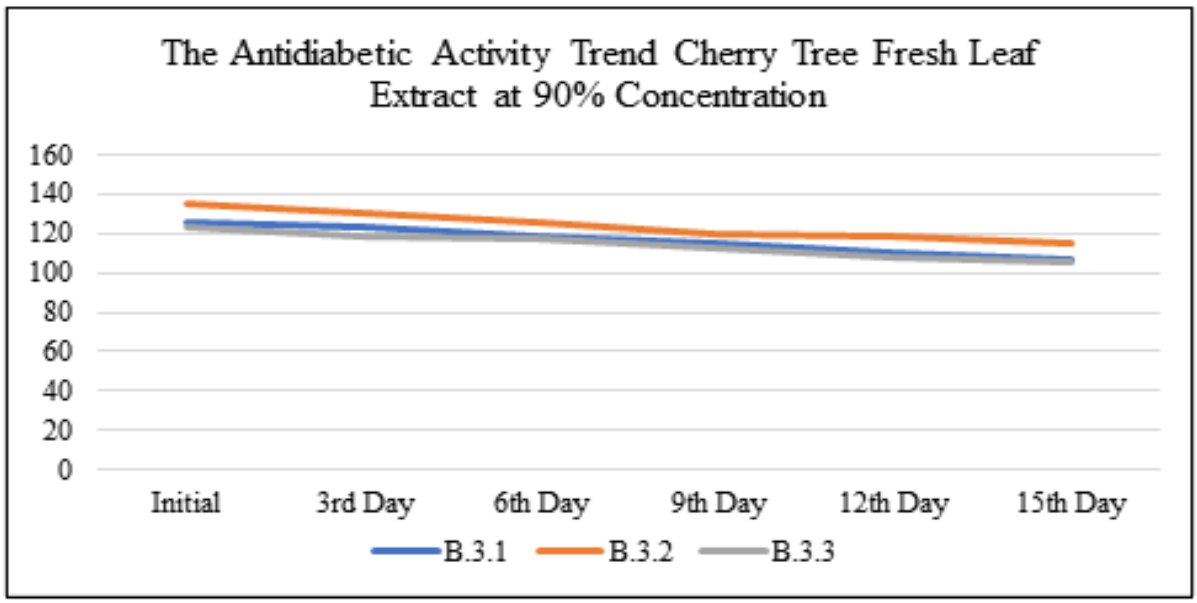

Fig. 8. Graph showing the anti-diabetic activity trend of Cherry Tree fresh leaf extract at 90% concentration.

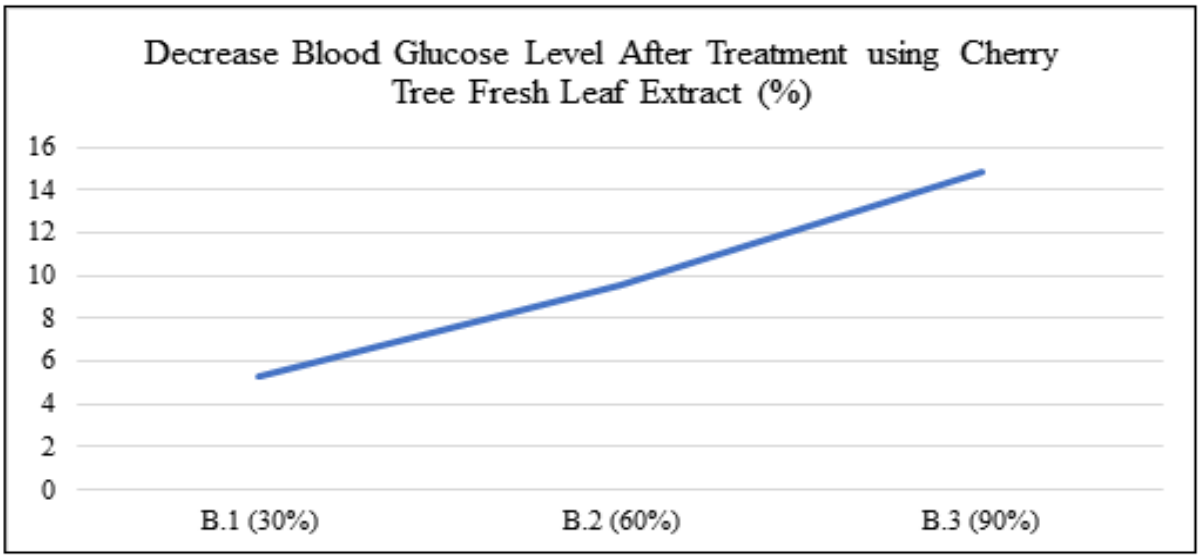

Fig. 9. Graph showing the increasing anti-diabetic activity of Cherry Tree fresh leaf extract as the fresh leaf extract concentration increases.

It is known that one of the major causes of the development of diabetes and other diseases is oxidative stress. Therefore, those plants that exhibit antioxidant activities can help cure diabetes and other diseases (Moradi *et.al.,* 2018). In the phytochemical screening of the Insulin Plant *(Costus pictus),* it was revealed that it is rich in protein, iron, and antioxidant components such as ascorbic acid, α-tocopherol, β-carotene, terpinoids, steroids, and flavonoids (Hedge, P.K., 2014). The same goes for the antioxidant activity of the leaf extract of Cherry Tree *(Muntingia calabura)*. In a study entitled Antioxidant Activity: Root, Leaves and Fruits Aqueous Extracts of Muntingia Calabura it was found that the leaf extract of Cherry Tree *(Muntingia calabura)* (52%) showed more antioxidant activity in comparison to fruit extract (25%) and the root extract (43%) showed less. The above antioxidant activity of the extracts may be due to the presence of phytochemicals like polyphenols, proteins, flavonoids, ascorbic acid and αtocopherol (Khan *et al.*, 2015). In the results mentioned above of different studies, it can be concluded that both Insulin Plant *(Costus pictus)* and Cherry Tree *(Muntingia calabura)* is an herbal

medicine that can cure diseases such as diabetes, for it contains different antioxidant components which are very helpful in lowering the blood glucose level.

Conclusion

The findings of the study signify that Insulin Plant (*Costus pictus)* and Cherry Tree (*Muntingia calabura)* fresh leaf extracts have anti-diabetic properties and thus can help lower blood glucose levels. There is no significant difference in the anti-diabetic activity of the fresh leaf extracts of the Insulin Plant and Cherry Tree at 30% concentration, but a significant difference in the anti-diabetic activity of the fresh leaf extracts of the Insulin Plant and Cherry Tree at 60% and 90% concentrations was found out after the experimentation process. The results revealed that as the concentration of the fresh leaf extracts both from the insulin plant and cherry tree increases, the dropping percentage of blood glucose level also increases which indicates that administering higher concentration of the insulin plant and cherry tree fresh leaf extract increases the effectivity in lowering the blood glucose level. From the results, it can be concluded that at 60% and 90% concentration, Insulin plant fresh leaf extract does have a greater percentage in decreasing blood glucose levels than the fresh leaf extracts of the Cherry Tree. Therefore, the Insulin plant and Cherry Tree are promising anti-diabetic alternatives that could be of great help in the field of medicine.

Conflicts of interest

The authors report no financial or any other conflicts of interest in this research.

Ethical approvals

This study followed highest ethical considerations before and after the conduct of the study. Albino Mice involved in this study were all alive and in good health status.

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