# LEAF EXTRACTS OF TWO RUBIACEOUS SPECIES (*MORINDA CITRIFOLIA L.* AND *IXORA COCCINEA L.*) AS AN AGENT IN HASTENING BLOOD COAGULATION TIME AND LOWERING BLOOD SUGAR LEVEL OF RATS (*MUS MUSCULUS*)

**(RESEARCH PAPER)**

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# INTRODUCTION

## Background of the Study

Blood is vital for it supply nutrients for the body to function. Being the main transport system of the body, it is important that one has healthy blood. However, there are many blood disorders which can affect one's self. 10,000 deaths came from hematologic disorders at the United States on 2010. These include anemia, blood coagulation defects, and purpura (National Heart, Lung, and Blood Institute, 2012). Another common example of such disorders is diabetes mellitus, which is the above normal increase of blood sugar due to either the malfunction of insulin production or inability of the body to respond properly with insulin. In the Philippines, diabetes affects 9.7% of the adult population on 2012 in which 11 million Filipinos are likely to have diabetes or prediabetes (Valisno, 2013).

Symptoms of the said disease include frequent urination, recurring thirst and hunger, and cuts and bruises that do not heal (Nordqvist, 2015). When a cut is not healed immediately, it is prone to fungal and bacterial infections. Meanwhile, in studies about the circulatory system, blood coagulation aids in curing wounds and cuts for the blood has fibrin and platelets which seals the wound (Laurens, Koolwijk, & de Maat, 2006). Therefore, the increased time of blood clotting means faster healing time. In search for solutions to such disorders and ailments, plants have long been known as an alternative medicine for wounds and injuries due to its phytochemical constituents and are proven as an affordable and safe type of medicine (Thakur, Jain, Pathak, & Sandhu, 2011). In

studies in biodiversity, such constituents are found in the Family Rubiaceae which exhibits properties like *antimicrobial, antimalarial, antioxidants* and *anti-inflammatory* activities (Karou, Tchacondo, Ilboudo, & Simpore, 2011).

*Morinda citrifolia* L. (Rubiaceae) is a small tree native to South Eastern Asia and Australasia. Its common names include noni, indian mulberry, ba ji tian, nono or nonu, cheese fruit, and nhau in different parts of the world. Apatot, widely-known term in the Philippines, was traditionally used to treat broken bones, deep cuts, bruises, sores, and wounds. It is also reported to have *antibacterial, antiviral, antidiabetic, antitumor* and *immunological* activities (Rasal, Sinnathambi, Ashok, & Yeshmaina, 2008).

Meanwhile, from the same family, *Ixora coccinea* L., (Rubiaceae) commonly known as santan in the Philippines, is an evergreen shrub native in South India and Sri Lanka. Plant parts of *Ixora coccinea* L. such as flower, leaves, roots, and stem are used to treat various ailments in India and in different folk medicines. According to pharmacological studies, the said plant possesses *antioxidative, antibacterial, gastroprotective, hepatoprotective, antidiarrhoeal, antinociceptive, antimutagenic, antineoplastic* and *chemopreventive* effect (Baliga and Kurian, 2012).

With the information gathered on the ability of the said plants as a remedy for blood coagulation and lowering blood sugar, very few literatures in the Philippines features the uses of these plants as an alternative. In this study, the general purpose is to

determine the blood coagulation time and effect of blood glucose from plant extracts of different species of Family Rubiaceae such as *Morinda citrifolia* L. and *Ixora coccinea* L.

**Statement of the Problem**

This study aims to determine the effects of *Morinda citrifolia* L. (apatot) and *Ixora coccinea* L. (santan) extracts in blood coagulation time and blood sugar testing as inferred from the *Mus musculus* (rats*)*. Specifically, this study intends to:

- obtain extracts from the leaves of *Morinda citrifolia* L. (apatot) and *Ixora coccinea* L. (santan);
- determine the blood coagulation time of apatot leaf extract in rats;
- determine the blood coagulation time of santan leaf extract in rats;
- determine the effects of apatot leaf extract to the blood sugar of rats; and
- determine the effects of santan leaf extract to the blood sugar of rats.

**Significance of the Study**

Determination of the study regarding the effects of *Morinda citrifolia* L. (apatot) and *Ixora coccinea* L. (santan) will be of great help to the different members of society:

*Household.* Through this study, members of the house would be encouraged to plant apatot and santan as an alternative source in treating and curing cuts and wounds. This may also provide remedy for people with high blood sugar.

*Medical professionals.* Using the natural potential of the said plants, a new study can be devised by modern medical and pharmaceutical industry as solution for specific conditions related to blood disorders.

*People with blood disorders.* The healing and preventive properties of the said plants for blood coagulation and lowering blood sugar may be of great information for people with hematologic disorders in curing their blood conditions.

**Scope and Limitations**

The primary focus of this study is to determine the blood coagulation time and the effect on blood sugar level in different plant extracts from Family Rubiaceae such as *Morinda citrifolia* L. (apatot) and *Ixora coccinea* L. (santan). Yet, proving apatot and santan as cure for any other blood clotting disorder or as cure for high blood sugar were beyond the scope of the study. In conducting this study, rats, from only one source, were used as test subjects for blood samples by means of an improvised fibrinogen test for blood coagulation time. The effects of the two plants in blood sugar were proven using point-of-care-testing (POCT) carried out with a blood glucose meter.

The creation and testing done in this study were conducted from June to September 2015 at Cavite National High School.

# METHODOLOGY

## Materials

Extraction of Plants

- *Morinda citrifolia* L. leaves or apatot
- *Ixora coccinea* L. leaves or santan
- mortar and pestle
- cheese cloth
- scissors
- gloves
- containers

Blood Coagulation

- Three *Mus musculus* or dagang costa
- extracts
- lancet
- lancing device
- 27 glass slides
- 27 toothpicks
- stopwatch timer

Blood Sugar Test

- Four *Mus musculus* or dagang costa
- extracts
- 29G syringe (needleless)
- D50 Water
- Easy Mate Glucometer or blood sugar tester
- lancet
- lancing device

**Procedure**

Extraction of leaves. *Morinda citrifolia* L. (apatot) leaves were rinsed with water. Leaves were cut into small pieces. Mortar and pestle were used in pounding the leaves. After pounding, the extracted juice was placed on a cheese cloth. Cheese cloth was then positioned over a container and the material was pressed manually. A strainer was used to separate bits and pieces from the juice. After straining, the extracted juice was placed on a clean container. The procedure was also repeated for the extraction of *Ixora coccinea* L. (santan) leaves.

Getting blood samples. Lancet was used to get blood samples at the lateral vein of a rat. After pricking of lancet in the vein, the blood was dropped on a slide then the fibrinogen test was performed. The drawing of blood was done three times each day (one blood drop without treatment; one with apatot; and another for santan), and were completed for three days. The same procedure was carried out to other two rats.

Fibrinogen testing. Once the blood emerges from the lateral vein, the timer will start. Using a toothpick, blood was gently stirred in an upward motion. As soon as fibrin appeared on the blood sample, timer was stopped. For Rat B and Rat C, the procedure was also repeated. After performing the test, data has been noted.

Preparation for blood sugar testing. Calibration of the glucometer was first done to check if the device is working. Sharp ends of the syringe were removed to ensure the safety of the rats. Another set of rats was used for this testing. Also, food and water were not given to all the rats during the testing. Each rat has been randomly assigned on four

setups: Rat 1 (normal and untreated); Rat 2 (diabetic and untreated); Rat 3 (diabetic and treated with apatot leaf extract); and Rat 4 (diabetic and treated with santan leaf extract).

Blood sugar testing. Four rats were tested for the intial testing. Three of the rats (Rat 2, 3, and 4) were administered with 0.5 ml of D50 Water using a 29G needleless syringe. After ten minutes, blood was drawn from the lateral vein of the four rats and placed on the test strips to obtain the baseline of their blood sugar level. After which, Rat 3 was induced with 0.5 ml apatot leaf extract while Rat 4 was induced with 0.5 ml santan leaf extract. After an hour, the blood sugar level of the four rats were checked and tested in the Easy Mate Glucometer. The blood sugar testing was done after the next two, four and six hours. After performing the experiment, the data was noted.

Data analysis. Mean was used for all the obtained data in the all the test performed.

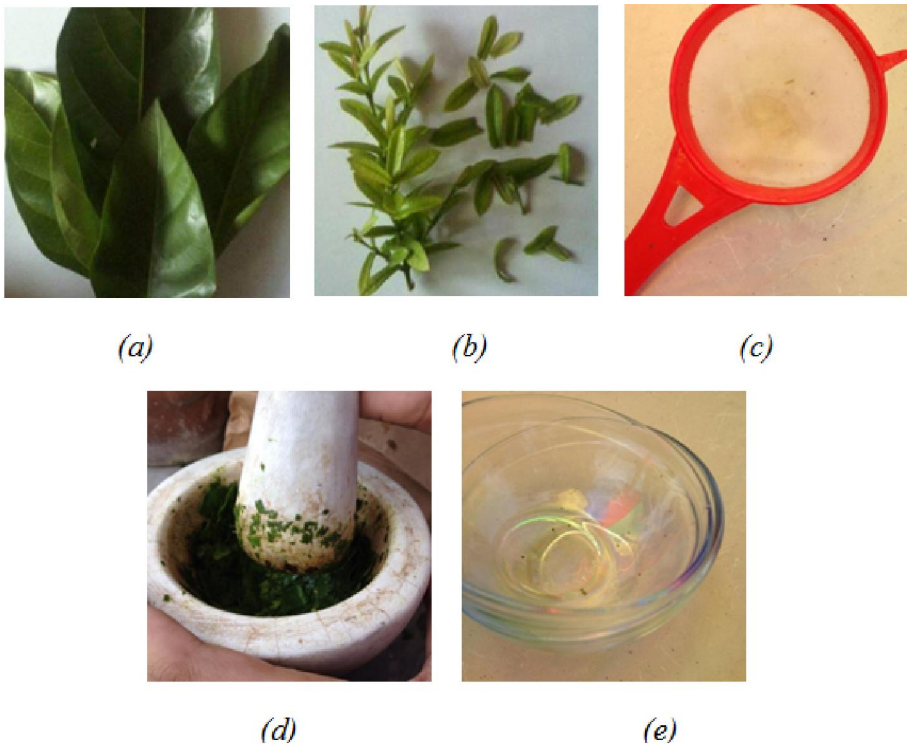


*Figure 1. Materials used for leaf extraction: (a) apatot leaves; (b) santan leaves; (c) strainer; (d) mortar and pestle; (e) containers*

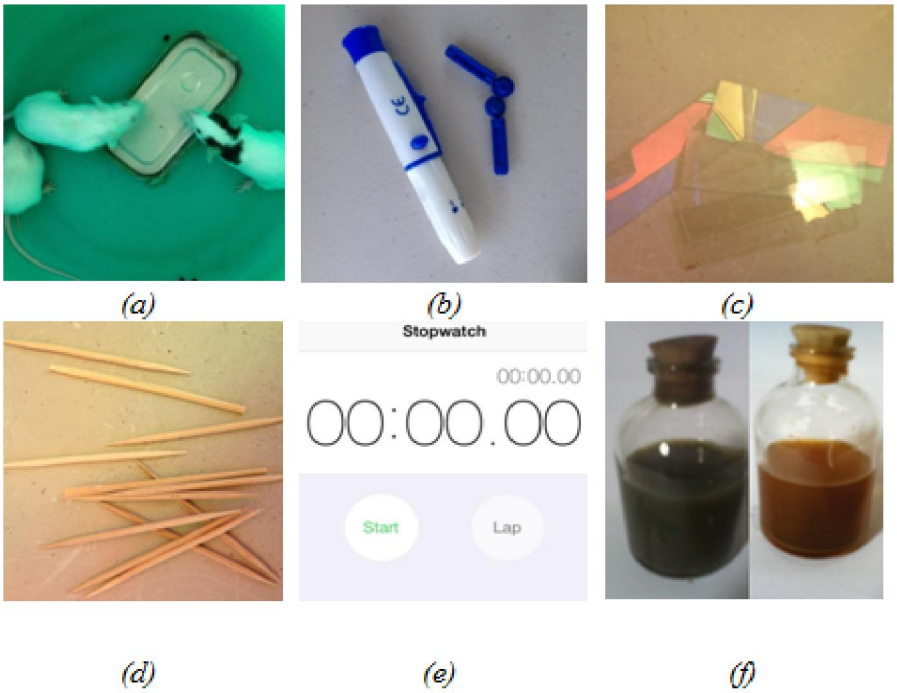


*Figure 2. Materials used for blood coagulation test: (a) Mus musculus* or dagang costa*; (b) lancet and lancing device; (c) glass slides; (d) toothpicks; (e) stopwatch timer; (f) extracts*

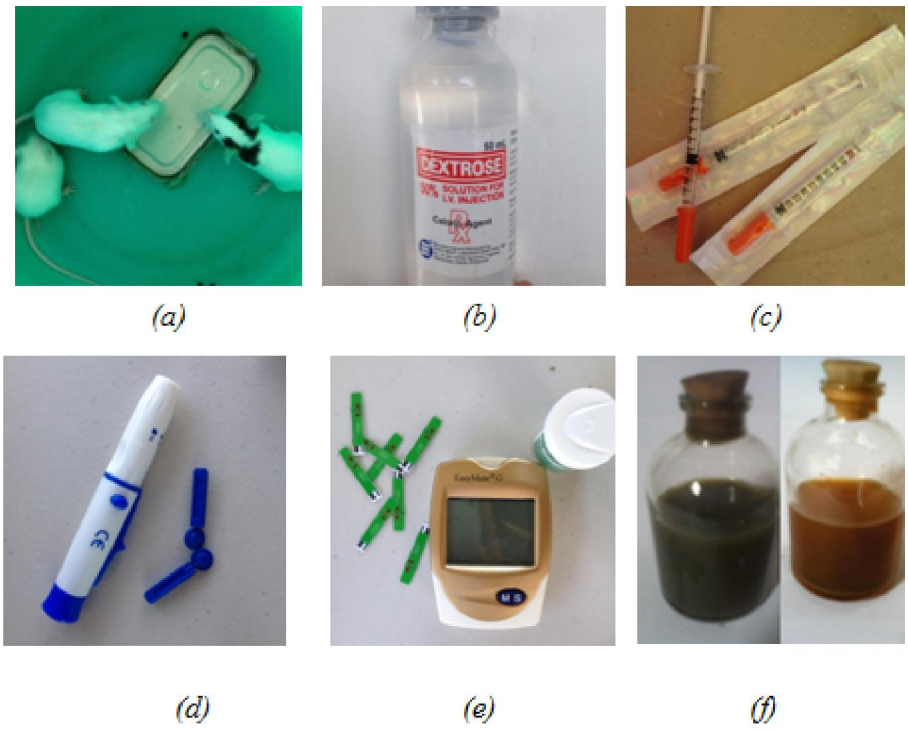


*Figure 3. Materials used for blood sugar test: (a) Mus musculus* or dagang costa*; (b)D5050 Water; (c) 29G needleless syringe; (d) lancet and lancing device; (e) Easy Mate Glucometerl (f) extracts*

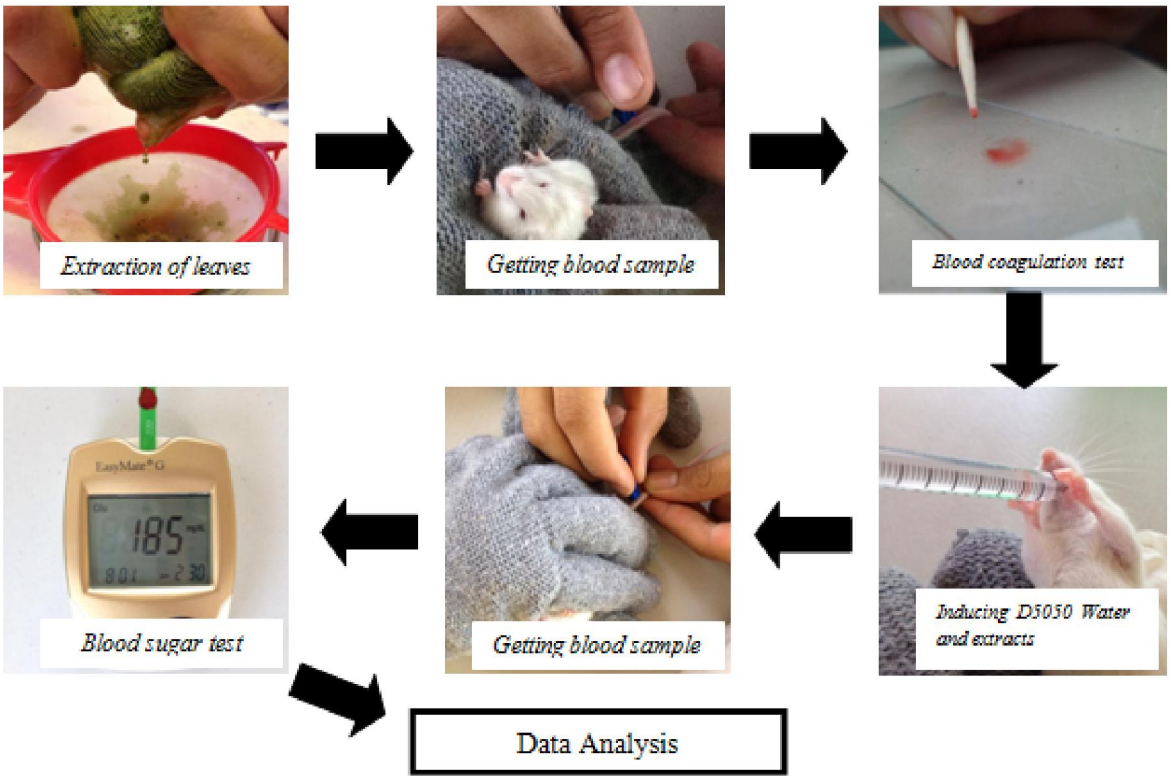


*Figure 4.Flowchart of extraction of leaves, blood coagulation test and blood sugar test*

# RESULTS AND DISCUSSION

## Leaf Extraction from *Morinda citrifolia* L. (Apatot) and *Ixora coccinea* L. (Santan)

Extracts were obtained from the cut and pounded leaves of apatot and santan. It is used for the blood coagulation test and blood sugar test. Following is the pictorial data showing the extracted material from the both leaves. (Figure 5 and 6)

*Figure 5. Morinda citrifolia L. leaf extracts*

*Figure 6. Ixora coccinea L. leaf extracts*

Figures above show the extracts of the two leaves. Apatot extract is in its dark green color with a strong scent. Meanwhile, Figure 6 shows the extracted material of santan, which has yellowish color and aromatic scent. However, apatot gives of more aromatic and stronger scent than santan.

## *Morinda citrifolia* L. (Apatot) and *Ixora coccinea* L. (Santan) as Blood Coagulant

After getting blood samples from rats, extracts of apatot and santan were dropped into the samples. Then, fibrinogen test was performed. Table 3 shows the blood coagulation time in different set ups.

From the testing done, it is evident that blood treated with *Morinda citrifolia* L. (Apatot) showed the fastest coagulation time (at 17.72 second average) among the other two setups. It was followed by the blood treated with *Ixora coccinea* L. (Santan) (with average of 27.54 seconds). The negative control group possessed the least anti-thrombolytic effect (with an average of 32. 57 seconds).

*Table 1. Blood coagulation time of blood samples in different set ups*

| Set up | Blood coagulation time of blood samples in every trials (seconds) | | | | | | | | | | | | Final Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RAT A | | | | RAT B | | | | RAT C | | | | |
| | 1 | 2 | 3 | $\bar{x}$ | 1 | 2 | 3 | $\bar{x}$ | 1 | 2 | 3 | $\bar{x}$ | |
| Treated with *Morinda citrifolia* L. leaf extracts | 28.49 | 15.33 | 23.91 | **22.58** | 19.22 | 9.31 | 15.21 | **14.58** | 10.73 | 17.21 | 20.12 | **15.99** | **17.72** |
| Treated with *Ixora coccinea* L. leaf extracts | 36.54 | 24.23 | 25.22 | **28.66** | 41.35 | 15.71 | 18.11 | **25.06** | 31.43 | 29.03 | 26.24 | **28.9** | **27.54** |
| Without treatment of extracts | 43.64 | 18.43 | 31.71 | **31.26** | 56.24 | 30.07 | 13.67 | **33.33** | 38.92 | 34.84 | 25.58 | **33.11** | **32.57** |

The measured values agreed well with the objectives of the study proving each of the plants as pro-coagulants. The most favorable response came from the results of *Morinda citrifolia* L. (Apatot) with a coagulation time almost half of the blood without treatment. The figure below shows the average coagulation time in different set ups.

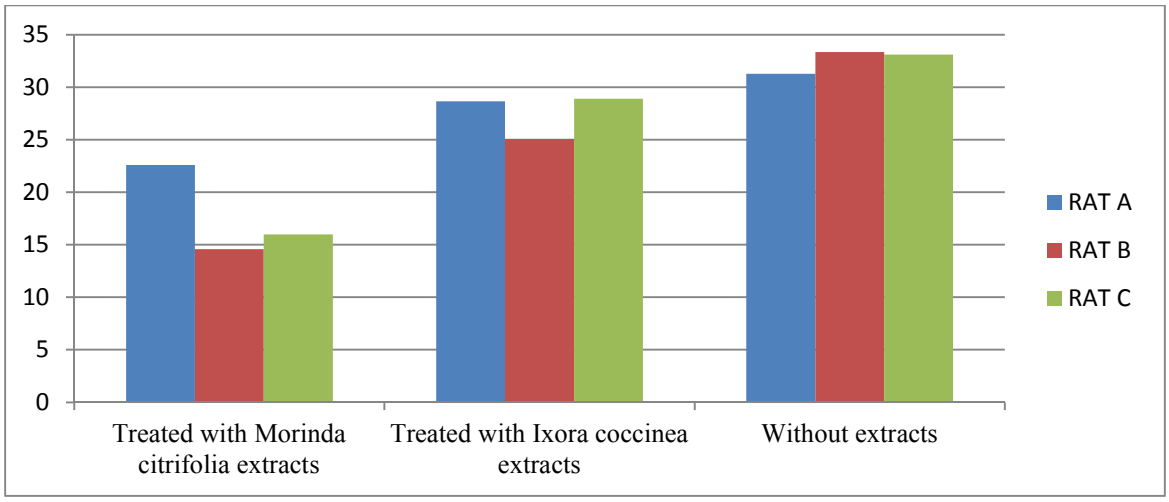


*Figure 7. The average coagulation time of blood samples in different set ups*

Figure 7 shows apatot and santan can be used as blood coagulant. Apatot has alkaloid, scopetin, polysaccharide, potassium, vitamin c, and terpenoids (Karim, Noor, Seman, Tohit, & Idris, 2013). And also, santan is used as wound healing herb for it has higher hydroxyproline content, and improved histopathology of granulation tissue that can help to fasten the wound contraction (Upadhyay, Aadesh; Chattopadhyay, Pronobesh; Goyary, Danswrang; Mitra Mazumder, Papiya; Veer, Vijay, 2014).

## *Morinda citrifolia* L. (Apatot) and *Ixora coccinea* L. (Santan) in Lowering Blood Glucose

Four rats were randomly assigned for four setups: Rat 1 (normal and untreated); Rat 2 (diabetic and untreated); Rat 3 (diabetic and treated with apatot leaf extract); and Rat 4 (diabetic and treated with santan leaf extract). Data for initial testing was gathered in all of the rats. D50 water was introduced to the three rats (Rat 2, 3, and 4) to increase blood sugar level. After ten minutes, blood sugar level of the four rats was testing. It was

then followed by the ingestion of extracts of apatot for Rat 3and extracts of santan for Rat 4. Blood sugar testing was done after the next two, four, and six hours.

*Table 2. Blood sugar levels every hour*

| Set ups | Trials (mg/dL) | | | | | |
|---|---|---|---|---|---|---|
| | Initial | Baseline | Aft. 1 hr. | Aft. 2 hrs. | Aft. 4 hrs. | Aft. 6 hrs. |
| 1 (Not Diabetic; Untreated) | 85 | 88 | 77 | 67 | 47 | 56 |
| 2 (Diabetic; Untreated) | 48 | 213 | 198 | 215 | 84 | 64 |
| 3 (Diabetic; Treated with *Morinda citrifolia* L.) | 120 | 293 | 117 | 116 | 98 | 85 |
| 4 (Diabetic; Treated with *Ixora coccinea* L.) | 73 | 195 | 74 | 69 | 50 | 44 |

Rat 1 has an initial blood sugar level of 85 mg/dL and has the baseline of 88 mg/dL, considering that it has not undergone inducing of D50 Water. After the succeeding hours, its blood sugar had gradually decreased from the baseline, 88 mg/dL, down to 56 mg/dL, after six hours.

Meanwhile, Rat 2 with 48 mg/dL for its normal blood sugar level had an intake of D50 Water. After inducing, its blood sugar level escalated up to 213 mg/dL and without taking any treatment, it has continued to remain high until the second hour (215 mg/dL). After four hours, it dropped to 84 mg/dL and in its sixth hour, it became 64 mg/dL which is still high if compared to its initial.

Rat 3, which started with a high blood sugar level of 120 mg/dL, was also administered with D50 Water resulting to an almost tripled initial. After inducing apatot

leaf extract, the blood sugar level has decreased from 293 mg/dL (baseline) to 117 mg/dL. Then, for the following hours, its trend of decrease continued down to 85 mg/dL.

Lastly, Rat 4 started with 73 mg/dL as its initial blood sugar level. Upon inducing D50 Water to the rat, its blood sugar level rose to 195 mg/dL. Then, after ingesting santan leaf extracts to the rats, the blood level declined 74 mg/dL after an hour. After the succeeding hours, its development continued to decrease down to 44 mg/dL.

It can be inferred from the data table that D50 Water was successful in increasing blood sugar level though it has varied results for each rat. It is also noticeable that after the forth hour, all of the setups' blood sugar level are lower than its initial blood sugar level. However, there is an exception with Rat 2 whose initial (48 mg/dL) is very low if compared to others.

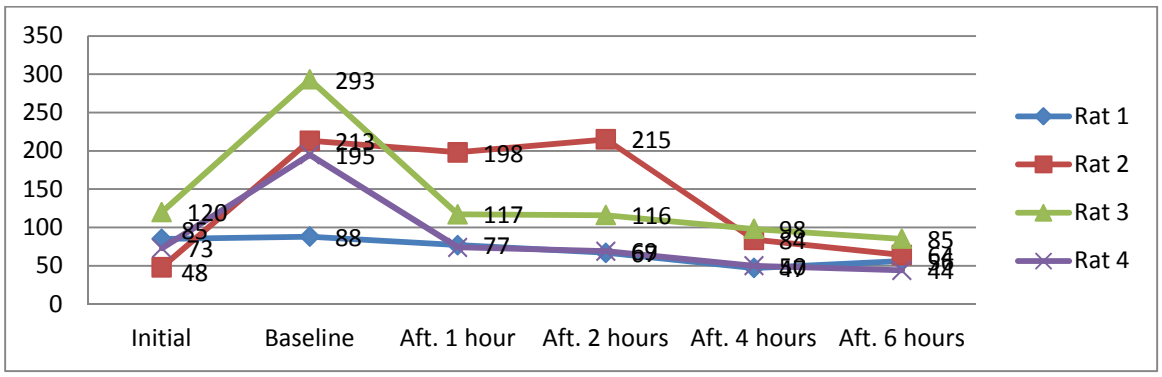


*Figure 8. Blood sugar level of blood samples in different set ups (mg/dL)*

From the testing done, it is evident that the blood of Rat 3 that is treated with apatot showed the highest difference in blood sugar level among the other three setups ( It was followed by the Rat 4 whose blood is treated with santan (with difference of 148 mg/dL after two hours; and 191 mg/dL after three hours). The negative control group

possessed the least hypoglycemic effect (with -14 mg/dL and 9 mg/dL after three hours). Figure 8 shows the blood sugar levels of the rats in different trials.

The result may be due to the effect of hypoglycemic herbs to treat diabetes by insulin sensitivity increasing insulin production and decreasing the amount glucose in blood (Lee, Park, Hwang, Yi, Nam, & Lim, 2012). The greater effectivity of *Morinda citrifolia* L. (Apatot) may be due to its ability to decrease the blood sugar level (Hui, Tang, & Go, 2009). Its efficiency can also be due to the presence of carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenol helped to *Ixora coccinea* L. (Apatot) to decrease the blood sugar level (Daske, Das, & Sharma, 2015).

## SUMMARY AND CONCLUSION AND RECOMMENDATIONS

The general purpose of the study is to determine the effect of plant extracts in blood coagulation time and blood glucose level of different species of Family Rubiaceae such as apatot (*Morinda citrifolia* L.) and santan (*Ixora coccinea* L.) on rats (*Mus musculus)*. Upon creation of extracts, the study intends to prove that both of the plants can quicken the formation of fibrin in blood and also lower the blood sugar level of the test subjects. Apatot and another with santan extracts were freshly prepared in the beginning of the experiment. Three rats were used as subjects for the Fibrinogen test which would determine the coagulation time. Three blood drops were taken from each rat every day for three days and were placed in a glass slide. One of the blood drops were mixed with extracts of apatot and another with santan leaving the last drop without any treatment. Once the blood was drawn from the rat, the time starts. Using a toothpick,

blood was stirred in an upward motion. Timer will be stopped as soon as a fibrin appeared. For blood sugar testing, the normal blood sugar level of four rats was gathered. All rats, except negative control group (Rat 1- normal; untreated) were induced with 0.5ml D50 water. After which, blood was drawn and placed on a test strip of a glucometer for the initial testing. Rat 3 ingested 0.5 ml apatot extracts while Rat 4 with 0.5 ml santan extracts. After one hour, the blood sugar levels of all rats were collected. Blood sugar level was taken from each test subject to the next two, four, and six hours and was compared to the first blood sugar reading without any extract intake. Mean was used for all the obtained data in both set-ups.

After experimentation and analyzing of data, results showed that both apatot and santan extracts has an anti-thrombolytic effect on the blood of the rats. However, blood drop with apatot showed a faster coagulation time than both santan and negative controlled group.

Same results were obtained in the blood sugar testing wherein both of the extracts established hypoglycemic effects. Apatot displayed greater hypoglycemic effects than santan and the two negative control groups. Both positive control groups managed to lower the blood sugar of the mice while the negative control groups stayed high throughout the next two hours experimentation.

As a summary, the data suggests that both of the plant extracts used from the Family Rubiaceae were proven effective in hastening blood coagulation time and

lowering the blood sugar. It is also to be taken note of, that among the two extracts, apatot has proven to have a larger effect in both of the set-ups than santan.

This study recommends further studies which include the following:

- Use alternative plants that can hasten blood coagulation time and lowers blood sugar level;
- Application of this study in human as subjects to test the effectivity of two plants in an individual.
- Creation of medical products using apatot and santan with relation to their ability to lower blood sugar and hasten blood coagulation time;
- Perform other methods in determining blood coagulation time. Use procedure that tests other factors of blood clotting time.
- Analysis of bioactive compounds of apatot and santan that could have causes their anti-thrombolytic and hypoglycemic effect.

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