

Potential of Calamansi (*Citrofortunella microcarpa*) Fruit Peels Extract in Lowering the Blood Glucose Level of Streptozotocin Induced Albino Rats (*Rattus albus*)

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Abstract—Calamansi or calamondin is very abundant and one of the sources of staple fruit juice in the Philippines. It is grown principally for its fruit juice, since it is widely known as good source of Vitamin C. However, the peels are thrown after the extraction of the juice. The medicinal use of the peel was still unknown to many Filipinos, thus this study focuses on the potential of calamansi peels in lowering blood sugar in streptozotocin induced Albino rats. Calamansi peels were dried, macerated, and the filtrate was subjected to rotary evaporator. The extract were diluted with distilled water and administered orally in Albino rats. Twenty Albino rats served as experimental animals. They are randomly assigned in two groups. The first group, or treatment 1, (10 animals) as the control wherein they only fed with rat pellets and drinking water. The second group-treatment 2 served as the experimental animals where calamansi peel extract solution was administered orally for the entire duration of the study. Baseline blood glucose, fasting blood sugar before Streptozotocin (STZ) induction and blood sugar after three days STZ induction of both treatments showed no significant result. Final blood sugar after five days of administration of the calamansi peel extract solution showed significant result. The result revealed that calamansi peel extract solution has the potential for lowering blood glucose in Albino Rats. This implies that calamansi peel extract solution could be used as herbal medicine to lower blood glucose.

Index Terms—Calamansi, Calamondin, streptozotocin, blood glucose

I. INTRODUCTION

Calamondin (*Citrofortunella microcarpa*) fruits or locally known as “*Kalamansi*” is widely cultivated in Philippines and is used as a condiment almost in every famous dish made in the Philippines. Only the pulp were squeezed and is needed, the peels are just thrown away. It belongs to the family Rutaceae. It is an intergenetic hybrid between a member of *Citrus reticulata* or “tangerine” and “kumquat” or *Fortunella japonica*. The calamansi tree has

a height of 3 to 5 meters high, and is erect, slender, densely branched close to the ground, slightly spiny, and bears broad-oval, dark green leaves on the surface, yellowish beneath, sweetly fragrant white flowers with 5 elliptic-oblong petals, and fruits that are round that is about 4.5 cm wide with very thin, aromatic peel with visible pores. The pulp have 6 to 10 segments that is colored yellow to orange, very juicy, seedless or with 1 to 5 small ovoid green seeds within. It has been known not only for its refreshing juices and flavors, but also for its medicinal uses. Calamansi fruit may be crushed and use to shampoo in hair or may be applied into scalp after shampooing for hair growth and relief of itching. “Calamondin” juice may be also applied and rubbed on mosquito bites to eliminate irritation and itching. It also bleaches freckles and clear up *acne vulgaris* if applied regularly. Juice is also taken orally as a cough remedy and is sometimes combined with pepper, to expel phlegm. It can also be diluted and drunk warm as a fecal softener. Distilled oil of the leaves can act as a carminative for having a volatile oil content of 0.90% to 1.06% [1].”

Research found that the leaves of the evergreen shrub of kumquat (*Fortunella japonica*) which is common in China, and belongs to the same family Rutaceae with Calamondin (*Citrofortunella microcarpa*) has hypolipidemic, hypoglycemic, and antioxidative effects [2]. Calamansi is richly cultivated in the Philippines, its average annual per capita consumption is as waste [3]. Citrus fruits such as calamansi, is utilized mainly for its pulp and juice, the rest of the fruit or the pressed pulp, covering of pulp segment, seeds, and the rind are considered largest source of citrus waste, finding a way to utilize it will also help the environment. Since calamansi belongs to citrus family it can now be utilized as source of herbal medicine. If its potential use as herbal medicine is explored it can also help in the reduction of environmental pollution.

Diabetes mellitus incidences increases daily and it is one of the top ten causes of death. In 2008, a survey was

conducted and showed that one in every five Filipino has diabetes. This only means 20% of the total population has diabetes. Although it has only increased 4% since 1998, these numbers still cause alarm since Filipinos diagnosed with diabetes are reported to be younger and younger [4]. In 2010, there were 285 million people worldwide diagnosed with diabetes. It is estimated to rise over 50% and incidence to increase to 438 million by 2030. Estimated 80% of diabetics live in developing countries [5].

With *Calamansi*, being common, well propagated, and has not been studied in Philippine setting with its hypoglycemic potential, hence this study. The result of the research will be useful to people who are prone to have diabetes mellitus, which in today's stressful lifestyle makes everyone at risk.

This study determines the baseline blood glucose, fasting blood glucose before streptozotocin induction, blood glucose on the third day after induction of streptozotocin and final blood glucose level after five days *calamansi peel* extract administration

Likewise the difference on blood glucose of the experimental group before and after *calamansi peel* extract administration was also evaluated.

II. METHODOLOGY

A. Materials

1) Experimental animals

Twenty Wistar Albino rats were used and were assigned in two groups. First group (10 rats-control), no *calamansi peel* extract in their diet. Second group (10 rats-experimental), 1.008 grams of *calamansi* peels extract per 1 ml of distilled water per rat. Rat cages with water dispenser, rodent feeds, purified water, improvised oral gavage and animal house with proper temperature was provided.

2) Calamansi peel extract

For *calamansi peel* extract preparation the materials used were the following; *calamansi* peels, oven, blender, 70% ethanol, storage jars, cheesecloth, Whatman paper number 1, rotary evaporator, distilled water.

3) For induction of streptozotocin

Streptozotocin 30 mg/kg per rat was administered It was dissolve in Citrate buffer to attain the pH of 4.5. Twenty tuberculin syringes with 24 gauge needle was provided.

4) Blood glucose monitoring.

For monitoring of blood glucose, sterile scissor, antiseptic, cotton balls and glucometer with strips was used.

B. Methods

1) Acclimatization of experimental animals

This study employed experimental research method, using twenty Wistar Albino rats, with an average weight of 126 grams. The rats were acclimatized for two weeks in an animal house. They were house individually, in standard cages for an acclimatization period of seven days or one

week before the commencement of experiment. During this period the animals had free access to standard pellet diet and water in *ad libitum* in an ambient temperature of (24 ± 2 °C); a standard laboratory condition [6]. They were housed according to the experimental lay-out as shown in Table I.

2) Induction of streptozotocin

Streptozotocin induces diabetes within 3 days by destroying the beta cells [7]. Each vial of sterilized Streptozotocin powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[[(methylnitro soamino)-carbonyl] amino]-D-glu copyranose and 200 mg. citric acid. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial is 3.5-4.5 because of the presence of citric acid. This material is prepared in 1-gr vials and kept in cold storage and refrigerator with temperature of 2-8 °C, away from light [8].

TABLE I. EXPERIMENTAL Layout

T1-control	T2- experimental
T1S1	T2S1
T1S2	T2S2
T1S3	T2S3
T1S4	T2S4
T1S5	T2S5
T1S6	T2S6
T1S7	T2S7
T1S8	T2S8
T1S9	T2S9
T1S10	T2S10

After one week of acclimatization. The rats were fasted for 12 hours. The fasting blood glucose was recorded. STZ induction was done patterned from the study on Antidiabetic Activity of Some Herbal Plants in Streptozotocin Induced Diabetic Albino Rats with lowered dosage [9]. STZ dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 30 mg/kg body weight and injected intraperitoneal, within 15 minutes of dissolution in a vehicle volume of 0.4 mL, with 1 mL of tuberculin syringe fitted with 24 gauge needle. (See Figure 1.). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin.

3) Preparation of Calamansi Peel extract

Calamansi was purchased from local market. Tap water was used to remove adhering dirt. The peels were removed manually. A study on Antimicrobial & Antioxidant Activity of Orange Pulp and Peel, with modification served as guide in oven drying [10]. The peels were oven dried for 15 minutes at 95 degrees centigrade until the peel became crispy for grinding (see Figures 2.a. and 2.b). It was grinded in an electric blender. It was macerated for three days following the study on the Preparation of Plant Extracts from Indigenous Medicinal Plants [11]. Three hundred grams of *calamansi* peels were macerated in 1200 ml of ethanol for three days with constant agitation. After three days, filtration was done using cheesecloth and

Whatman paper number 1 (See Figure 2c.). The filtrate was subjected to rotary evaporator for 3 hours (Figure 2d.). After which, the crude extract was subjected to double boiler for the removal of the remaining ethanol. The solid like product was stored in the refrigerator (See Figure 2e.).



Figure 1. Induction of Streptozotocin



Figure 2 a. Calamansi Peels during oven drying



Figure 2 b. Calamansi peels after it was oven dried



Figure 2 c. Maceration of Calamansin peels in 70% ethanol.



Figure 2 d. Filtrate of Calamansi peels in rotary evaporator



Figure 2 e. Calamansi peels extract

4) Monitoring of blood glucose

Collection of blood samples were done to monitor the following; baseline/initial blood glucose, fasting blood glucose, blood glucose after three days STZ induction and final blood glucose after five days of *calamansi* peels extract administration. Blood samples were collected from the tail end. It was snipped using sterile, sharp surgical scissors (no more than 2 mm in rats) for blood collection [9] (see Figure 3a.). Afterwards, the second drop of blood was placed on the glucose strip and was analyzed using the glucose meter (Figure 3b.).



Figure 3 a. Tail snipped of Albino rat



Figure 3 b. Blood sample in glucometer

5) Administration of Calamansi peel extract

The *calamansi* peels extract was administered on the third day after streptozotocin induction where rats became hyperglycemic. Control group (T1) had an average of 122.9 mg/ dl of blood glucose, and experimental group (T2) had 116.7 mg/dl of blood glucose. The rats were given *calamansi* peels extract at 200 mg/kg of body weight [10]. Thus each rat (with an average weight of 126 grams) received 1.008g of extract mixed in .9 ml of distilled water for 5 days, It was administered orally by the used of improvised gavage as shown in Figure 4.



Figure 4. Administration of calamansi peels extract

C. Data Gathered and Statistical Tools

Data gathered are the following:

1. Baseline sugar level/initial Blood glucose
2. Fasting blood glucose
3. Blood glucose after three days STZ induction
4. Blood glucose after Five days *calamansi* peels extract administration.

The data gathered was statistically evaluated through standard T-test. A statistical analysis was performed using Microsoft Excel, version 2013. A significant difference was achieved when the value of t-statistics is greater than t-critical value.

III. RESULTS AND DISCUSSION

A. Baseline Sugar Level before Fasting of the Albino Rats

Table II shows the baseline blood glucose level of both Treatment 1 and Treatment 2 before the induction of Streptozotocin. Treatment 1 (control) had an average blood glucose of 98.2 mg/dL. Treatment 2 had an average blood glucose of 100.1 mg/dL. T-test was used to determine the significant difference between the treatment groups and no significance was shown in this table, which signifies that all experimental animals were treated equal in the beginning of the study to avoid bias to the result.

B. Blood Glucose after Twelve Hours Fasting - before STZ Induction

Fasting blood glucose level of both Treatment 1 and Treatment 2 before the induction of Streptozotocin is shown in Table III. Treatment 1 (control) had an average blood glucose of 122.7 mg/dL. Treatment 2 had an average

blood glucose of 116.9 mg/dL. T-test showed no significant difference between the two treatments.

C. Blood Sugar Level of the Albino Rats after Three Days Stz Induction

Table IV shows the blood glucose level of both Treatment 1 and Treatment 2, three days after the induction of STZ. Treatment 1 (control) had an average blood glucose of 122.7 mg/dL. Treatment 2 had an average blood glucose of 116.9 mg/dL. T-test revealed no significant difference between the two groups. It implies that the blood glucose level of the two groups after three days STZ induction is similar and that blood glucose level are treated equal after three days STZ induction.

D. Final Blood Sugar Level of the Albino Rats

Table V exhibits the final blood glucose level of both average blood glucose of 103.9 mg/dL. Treatment 2 had an average blood glucose of 75 mg/dL. T-test was used to determine the significant difference between the treatment groups. It shows significant difference in the blood glucose levels of the sample of Treatment 1 and Treatment 2. The result indicate that blood glucose level of STZ induced rats (T2) significantly respond to the effect of flavonoids found in *calamansi* peel extract. Several studies supports the claims in this study.

A study on flavonoid content of selected citrus Fruit Rind which shows that the *calamansi* extract yielded the highest flavonoid concentration which is 487.72 mg/L; followed by the lemon extract at a flavonoid concentration of 447.80 mg/L.[12]. Another study on the flavonoid compositions and antioxidant activity of *calamondin* extracts prepared using different solvents showed that total phenolic and flavonoid contents of extracts from peel of *calamondin* were higher than that from pulp, except the flavonoid content in hot water extract. The flavonoids found in extracts of *calamondin* were

0,50-di-C-b-glucopyranosylphloretin (DGPP), naringin exhibited the highest quantity, while naringin and hesperidin were the other two major flavonoids [13].

Citrus peel extract containing polymethoxy xylated flavones (PMFs) may help prevent diabetes [14]. The pectin of the orange peel (as a natural fiber) can decrease the rise in blood sugar that may occur after a meal [15]. Citrus peels extract especially *calamansi* extracts maybe another great nutraceutical products that could be important in management of diabetes [16].

A study on the antidiabetic property of Citrus stem bark of the plant showed the presence of flavonoids and tannins in ethanolic extract. It is well known that flavonoids and tannins possesses antidiabetic property [17].

TABLE II. BASELINE BLOOD GLUCOSE LEVEL

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Ave.	T-Test
T1 (Control)	117	104	102	98	99	94	85	98	93	92	98.2	T Stat = 0.56 ^{ns} < t Crit = 1.73
T2 (with peel extract)	112	103	101	91	92	95	99	100	106	102	100.1	

TABLE III. BLOOD GLUCOSE LEVEL AFTER TWELVE HOURS FASTING-BEFORE STZ INDUCTION

Treatment	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Ave.	T-Test
1 (Control)	146	119	117	114	116	127	133	133	114	108	122.7	T Stat = 0.76 ^{ns} < t Crit = 1.73
2 (administered with calamansi peel extract)	115	108	104	94	159	111	149	122	98	109	116.9	

TABLE IV. BLOOD GLUCOSE LEVEL THREE DAYS STZ INDUCTION

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Ave.	T-Test
T1 (Control)	146	119	117	114	116	127	133	133	114	108	122.7	T Stat = 0.76* < t Crit = 1.73
T2 (with peel extract)	115	108	104	94	159	111	149	122	98	109	116.9	

TABLE V. BLOOD GLUCOSE (MG/DL) AFTER FIVE DAYS ADMINISTRATION OF CALAMANSI PEELS EXTRACT

T2	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Ave.	t-TEST
Initial	115	108	104	94	159	111	149	122	98	109	116.9	T Stat = 3.78* < t Crit = 1.73
Final	65	76	71	92	73	75	48	62	94	94	75	

E. Blood Sugar Difference (Final-Initial) in Experimental Groups (T2).

Table VI displays the difference between the final, and initial blood glucose level of the experimental group (T2). Having average of 116.9 mg/dL as their initial blood glucose level, it was decreased to an 75 mg/dL as the rat's average blood glucose level after 5 days (final blood glucose level) of introduction of the extract of *Citrofortunella microcarpa* peels. The T-test showed significant difference on the blood glucose level of the experimental rats, which further shows that the peel extract of *Citrofortunella microcarpa* reduced the blood glucose level of the experimental rats after 5 days of administration.

A study on the methanolic extract of sweet lemon showed similar result. It expresses that the methanol extract of the fruit peels of *Citrus limetta* or commonly known as "Sweet Lemon" also belong to the family *Rutaceae* along with *Fortunella japonica* (Kumquat) and *Citrofortunella microcarpa* (Calamansi), has great effect in lowering the blood glucose level activity against STZ-induced diabetes

as well as hypoglycemic activity in normoglycemic rats in hypoglycemic rats [10]. The citrus plants are known to be rich in flavonoids which are polyphenolic compounds having antioxidative property. These properties of the *Citrus limetta* peel are brought about by flavonoids that it contains. Flavonoids contained in the *Citrus limetta* peel such as hesperidin and naringin are both proven to be potent hypoglycemic agents, and their blood glucose-lowering property is postulated to be partly conveyed by changes in hepatic glucose regulating enzyme activities in db/db mice, in which the increased hepatic glucose production, added with decreased hepatic glycogen synthesis and glycolysis are the major symptoms in Type 2 diabetes that leads to hyperglycemia. These would be the consequence of the low glucokinase activity, glucose-6-phosphatase and Phosphoenolpyruvate carboxykinase (PEPCK) activity in diabetic state. One of the most sensitive indicator of glycolytic pathway in DM is hepatic glucokinase in which if increased, there will also be increased utilization of blood glucose. [18].

TABLE VI. BLOOD SUGAR DIFFERENCE (FINAL-INITIAL) IN EXPERIMENTAL GROUPS (T2)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Ave.	T-Test
T 1 (Control)	117	86	96	93	105	118	140	107	79	98	103.9	T Stat = 3.93 ^{n.s} < t Crit = 1.73
T 2 (with peel extract)	65	76	71	92	73	75	48	62	94	94	75	

IV. CONCLUSION AND RECOMMENDATION

There are numerous studies on the use of fruits from Citrus family in lowering blood glucose. However, the use of Calamansi peels is not yet explored. Findings of this study revealed that *calamansi* (*Citrofortunella microcarpa*) peels extract has hypoglycemic action in streptozotocin induced Albino rats and the effect was found significant five days after administration. With its effect in lowering blood glucose level comes a potential hypoglycemic effect. The findings of this study revealed that the peels of *Calamansi* can now be utilized as source of herbal medicine, thus maximizing the potential use of all parts of the fruit. Further studies should be explored on the possibilities of using other means in decreasing blood sugar level using *calamansi* peel extracts in different concentrations and different types of administration.

ACKNOWLEDGMENT

The authors acknowledge their invaluable administrators, from San Beda College, College of Arts and Sciences. Dr. Christian Bryan Bustamante- Dean and Dr. Moses Aaron Angeles-Vice Dean, for their constant encouragements. Special credits to Dr. Eduardo Lorico for his assistance in handling laboratory animals and Dr. Pacifico Calderon for the use of Animal House in the entire study.

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