Verification of antidiabetic potential of Aloe vera: α -amylase inhibitory assay of crude aloe gel and aloe latex with rind extracts

Deanne Alcalde[1], Diane May Tajo[1] and Dyan Reizl Valencia[1]

[1] *Philippine Science High School Western Visayas Campus - Bito-on, Jaro, Iloilo City 5000, Department of Science and Technology, Philippines*

Abstract – Aloe vera has been known to have antidiabetic potential. Some studies state that aloe vera is antidiabetic because of the aloe latex while some studies state that its antidiabetic property is exhibited by the aloe gel. This study aims to verify whether aloe vera latex with rind and aloe vera gel indeed have antidiabetic potential. This study used the -amylase assay in which the inhibition of maltose was measured using a spectrophotometer. The results show that both aloe gel and aloe latex with rind extracts have antidiabetic property. It is recommended that active antidiabetic constituents be isolated and undergo further analyses to determine which constituents have better potential to be used for drug development.

Introduction. – Aloe vera is widely grown worldwide and has many uses. It is used to treat burns, inflamed skin condition, diabetes, rheumatic arthritis, rheumatic fever, ulcers and indigestion. It is also used in treating inflamed internal organ conditions such as the inflammation of stomach, small intestines, liver, pancreas and kidneys (Joseph and Raj 2010). Diabetes, on the other hand, is a condition in which the process of food for use as energy is not properly done by the body. The body obtains its energy from consumed food which is processed into glucose or sugar. The hormone that helps glucose enter into cells of the body is known as insulin, a hormone which is produced by the pancreas. When one has diabetes, the body is either incapable of using its own insulin properly or it doesnt make enough insulin. This causes sugar to build up in the blood and increase the blood glucose level. Increased blood glucose level gives higher risk of getting diabetes; lowering blood glucose levels is one method to treat diabetes.

Aloe vera has a potential to treat diabetes according to a study of Kim et. al. (2009) as aloe vera showed significant results in decreasing insulin resistance and thereby lowering blood glucose levels of diabetic mice. Previous study by Yongchaiyudha et. al (1996) also shows that aloe vera has a potential to be a treatment for diabetes. This study gave oral administration of aloe vera juice to patients with diabetes for two weeks and results show that blood glucose levels and triglyceride levels fell.

In a study of Okyar et. al. (2001), they tested the effects of aloe gel extract and aloe leaf pulp extract on diabetic mice. The results show that aloe vera leaf pulp extract results to an increase in blood glucose levels of mice and only aloe vera gel showed a decrease in the blood glucose levels of the test samples. Thus, according to Okyar's et. al. (2001) study, aloe vera leaf gel has a potential to treat diabetes while aloe vera leaf pulp extract does not.

Ajabnoor (1990) was able to identify the active constituents of aloe vera barbaloin and isobarbaloin (collectively known as aloin) and resin. Tanaka et. al. (2006) also identified that the active diabetic constituents in aloe vera are the five phytosterols - lophenol, 24 - methyllophenol, 24-ethyl-lophenol, cycloartanol and 24- methylene cycloartanol. Phytosterols are found in the aloe gel while the aloins and the resin are found in the aloe latex. Thus, according to Ajabnoor (1990) the antidiabetic potential of aloe vera is due to substances in aloe latex, a yellowish substance between the aloe gel and aloe rind; and according to Tanaka et. al. (2006), the antidiabetic potential of aloe vera is due to its aloe gel.

Various studies state different results on the antidiabetic part of aloe vera; some studies show that it is due to aloe gel while some studies show that it is due to aloe latex or aloe leaf (rind). There is inconsistency in the results of various studies. Thus, there is a need to determine whether the aloe vera parts indeed antidiabetic potential.

This study was able to verify the capability of aloe vera parts - aloe gel and aloe latex with rind - to have antidiabetic potential. An in-vitro test,α-amylase inhibition assay was used to determine whether the aloe vera part positively or negatively inhibited sugar breakdown.

Materials and Methods. – *General Extraction of Aloe Vera Plant.* The leaves were separately plucked from the aloe plant. The leaves were then washed and tap dried. The aloe gel was separated from the latex and rind by scraping using a stainless steel spoon. The aloe gel extracts were immediately subjected to further processes while the aloe latex with rind extract were set aside and air dried for around 48 hours.

Antidiabetic Aloe Vera Constituents. The target constituents from the aloe gel are aloins and aloeresins while the target constituents from the aloe latex and rind are phytosterols. The following procedures intended on optimizing the crude aloe vera extracts to obtain refined concentrations of the target constituents.

Solvent-solvent Extraction. Aloe gel and aloe latex with rind extracts used methanol as the extracting solvent as target constituents are polar molecules. Nonpolar solvent used was hexane.

Optimization of Aloe Gel. The fresh leaf gel was homogenized using an electric blender. The aloe gel was mixed with 100 mL hexane and 100 mL methanol and placed in the separatory funnel. The mixture was shaken three times and the inverted stopper was removed to release air. The system was closed again and the shaking procedure was repeated 3-5 times. The mixture resulted to two immiscible solutions of methane extract (bottom layer) and hexane extract (top layer). Methanolic extract was retrieved and placed in a rotary evaporator. A more concentrated aloe gel and methanolic mixture was retrieved from the rotary evaporator.

Optimization of Aloe Latex with Rind. The latex and the rind was sun/shade dried for two days. The dried portions were manually broken to smaller pieces using a mortar and pestle and were further powderized using an electric blender. The aloe latex solutes were dissolved in 100 mL hexane. The solution was mixed with 100 mL methanol and placed in the separatory funnel. The mixture was shaken three times and the inverted stopper was removed to release air. The system was closed again and the shaking procedure was repeated 3-5 times. The mixture resulted to two immiscible solutions of methane extract (bottom layer) and hexane extract (top layer). The methanolic solution containing the aloe latex and rind was placed in a rotary evaporator. More concentrated aloe latex, rind, and methanolic mixture was retrieved from the rotary evaporator.

The aloe gel was tested by the assay first, then the latex and rind were tested by the alpha amylase test two days after. The same aloe plant was the source of the aloe gel and aloe latex with rind.

α-Amylase Inhibitory Assay. Aloe vera extracts with different concentrations were first prepared in test tubes we had 0%, 20%, 40%, 60%, 80%, and 100% by volume. The diluting agent used is distilled water. The 0% sample had no aloe vera extract and only distilled water. Total volume of the different concentrations was 2 mL. 200μL of 0.02 M sodium phosphate buffer, and 20μL of α-Amylase was added in the assay. In a concentration range of 10-100μL/ml, this solution was incubated at room temperature for 10 minutes. 20μL of 1% starch solution was added on each test tubes. 400μL of 3,5 - dinitro salicylic acid (DNSA) reagent was added. The tubes were incubated in boiling water for five minutes and cooled at room temperature. The reaction mixture was diluted with 5 mL distilled water. The absorbance was measured at 540 nm using a UV-spectrophotometer. The control used is the blank sample and reference concentration is Acarbose, an α-amylase inhibitor.

Statistical Analysis. The α-amylase inhibitory activity was calculated. The formula for percent inhibition was used:

$$\%Inhibition = \frac{(A_{540}control - A_{540}test)}{A_{540}control} * 100 \qquad (1)$$

The percent inhibition values were plotted versus log inhibitor concentration and was evaluated by nonlinear regression analysis from the mean inhibitory values using Microsoft Excel. The IC50 values were then determined from the plot.

Results and discussion. – α-Amylase inhibitory activity of *Aloe vera* spp. were investigated in this study. Methanolic extracts of aloe gel and aloe latex with rind were prepared and five different concentrations of each methanolic extract were tested for the percent inhibition of α-amylase. The percent inhibition of the samples and the IC50 values of the extracts were calculated.

Aloe latex with rind extract at 60% concentration showed highest inhibition activity (67.61%) while the aloe gel extract at 20% showed the least inhibition activity (-5.85%). The IC50 values are 39.12% and 155.82% for aloe latex with rind and aloe gel extracts respectively.

Since both the extracts were generally able to get positive percent inhibition results, it can be said that: in aloe vera, both the aloe gel extract and aloe latex and rind extract has the potential to inhibit the -amylase enzyme.

Table 1: Percent Inhibition and IC50 values of different concentrations of aloins and phytosterols.

conc. of extract (%)	**% inhibition of Aloins** (%)	**% inhibition of Phytosterols** (%)
20	33.62	-5.85
40	57.51	11.29
60	67.61	9.65
80	-	19.67
100	-	38.14

Table 2: IC50 values of aloins and phytosterols.

IC50 of of Aloins (%)	**IC50 of Phytosterols** (%)
39.12	155.82

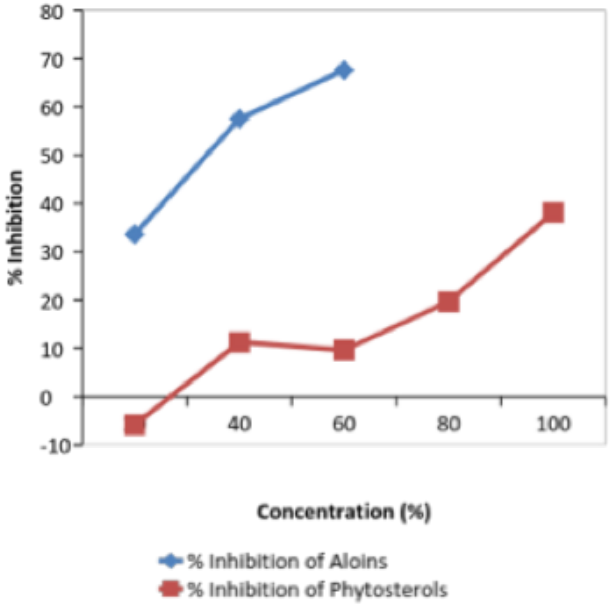

Fig. 1: The percent inhibition of aloins and phytosterols at different concentrations

It can also be observed in Figure 2 that as the concentration increases, the inhibition activity increases. This implies that the more the aloe extract, the greater the inhibition of the amylase enzyme.

However, the results are inconsistent as there are fluctuations in the percent inhibition as seen on the 20% and 40% aloe gel extract concentration. This might be due to an unhomogenized test samples, difference in optimization rates, and inconsistencies in the spectrophotometer.

The percent inhibition was calculated from the absorbance of samples in the spectrophotometer. The absorbance at 540 nm measures the amount of maltose in the sample. Each of the samples contains αamylase enzyme, sodium phosphate buffer, starch solution, dinitrosalicylic acid (DNSA), and the extract concentrations. The enzyme is responsible for breaking down the starch in the solution and the buffer maintains the pH of the solution so as to prevent any disruptions in the reaction. The broken down starch is maltose and some other substituents. The spectrophotometer measures the amount of light for a certain colour; however, the samples have vague colours. So, DNSA is needed for colouring the samples so that the target constituent could be read by the spectrophotometer.

The goal of this assay is to inhibit the enzyme activity of the α-amylase so the samples should have smaller amounts of broken down starch or maltose. Our reference sample is acarbose, a known α amylase inhibitor. Since acarbose is a known αamylase inhibitor, it would mean that the amount of maltose in the acarbose sample is zero or minimal because it was able to inhibit the αamylase enzyme, the responsible for making maltose. In the negative control sample, the extract was replaced with distilled water so there is no extract that could inhibit the activity of the enzyme and so the maltose level should be higher than the inhibitor. Expected absorbance values, output by spectrophotometer, should be lower than the negative control and at par with the positive control (or the reference sample) to say that the extract has a potential to inhibit the αamylase enzyme activity.

To be able to simplify the data, percent inhibition of each sample is calculated. Percent inhibition is a ratio of the difference between negative control absorbance value and sample value and the negative control absorbance value multiplied to 100%.

Antidiabetic activity is present once percent inhibition is positive. Higher percent inhibition means lesser amount of maltose present comparing it to the negative control. Positive percent inhibition is already an indication that the extract was able to inhibit antidiabetic activity being the one to lessen the amount of maltose in the sample.

The highest percent inhibition calculated is the aloe latex with rind extract at 60% concentration and the value is 67.61%. This would mean that it was able to inhibit 67.61% of enzyme activity compared to the negative control or the amount of maltose it had is 67.61% less compared to the negative control. The least inhibition calculated is the aloe gel extract at 20% concentration and the value is - 5.85%. This would mean that enzyme activity was not inhibited by the extract and that the amount of

maltose it had was 5.85% higher than that of the negative control.

Through the positive percent inhibition, this study was able to verify the antidiabetic potential of the two extracts aloe gel and aloe latex (and rind). The gel extract contains the phytosterols while the latex and rind extract contains the aloins. This study can be supported by studies of Ajabnoor (1990) and Tanaka et.al. (2006) that states that aloins and phytosterols found in aloe vera have antidiabetic potential.

To be able to represent the antidiabetic activity of the extracts, the IC50 values were calculated. The IC50 value is the concentration needed to be able to get a 50% percent inhibition. The IC50 values are 39.12% and 155.82% for aloins and phytosterols respectively. Positive IC50 values indicate that both aloins and phytosterols inhibit antidiabetic activity.

To be able to do this study or this method again, there are some key points that the researchers in this study learned. (1) The aloe gel and aloe latex should come from the same aloe vera plant to have more reliable results. (2) In the extraction of the extracts in the rotary evaporator, aim for the powdery extract and make sure to retrieve all of the solvent.

This study retrieved liquid extracts at the rotary evaporator so it had variable concentrations of methanol and aloe extracts. This study used the volume/volume percent concentration with extract from the rotary evaporator as the initial liquid and distilled water as the diluting agent. The ideal concentration units, basing Beer-Lamberts law, is mg/ml or μg/ml, so that the results will be more reliable. (3) Reference cell is the acarbose and this is needed when using the spectrophotometer; and control is the negative sample or the blank sample without any extracts.

The data gathering of this study was repeated twice because the first data gathering did not have the acarbose and the aloe extracts were collected from different plant sources. (4) Assay should have three replicates with three triplicates each to be able to have more reliable results. In this study, only one replicate with three triplicates was performed because of insufficient extracts. However, there were also inconsistencies with the triplicates. In the aloe gel methanolic extract, trial 1 was inconsistent with trials 2 and 3, and therefore it had been omitted in calculating the average of the percent inhibition of the phytosterols. Inconsistencies of these trials could be attributed to the samples which easily become unhomogenized even after shaking.

If ever this study will be repeated for verification, certain aspects can be improved especially on the data gathering procedure. More plots or more concentrations of samples can be done to increase validity and reliability of results. Replication of samples can be done for the assay to see if absorbance results vary and also get more reliable results. In performing the assay, one should note the proper storage conditions and concentrations of the chemicals. One should also note the placement and distribution of the chemicals during the assay and make sure that the solutions to be placed in the spectrophotometer are homogenous.

In summary, this study was able to show that both the latex and rind extract was able to inhibit the alpha amylase activity and that the extracts have a potential to be antidiabetic.

Conclusion. – Both aloe latex with rind extracts and aloe gel extracts, which are crude extracts of antidiabetic constituents aloins and phytosterols, respectively, have antidiabetic potential.

* * *

The researchers would like to recognize their advisers, Mr. Edward C. Albaracin and Mr. Ramon Angelo N. Sinco for their continuous support during the conduct of this study. They would also like to acknowledge the University of San Agustin Mendel Research Laboratories for accommodating the research data gathering.

REFERENCES

[1] AFIFI AF, KAMEL EM, KHALIL AA, FOAAD MA, FAWZIAND EM, HOUSENY M. Purification and characterization of a-amylase from peni cilliumolsonii under the effect of some anti oxidant vitamins. Global Journal of Biotech- nology and Biochemistry 2008;3(1): 1412.

[2] AJABNOOR MA. Effect of aloe on blood glucose levelling normal and alloxan diabetic mice. Jo urnal of Ethnopharmacology 1990;2:215-220

[3] ANABOLICMINDS.COM [Internet]. 2003. Beginners Guide to Soxhlet Extraction (Nov 2015). Available from: https://www.erowid.org/archiv e/rhodium/pdf /soxhlet 4dummies

[4] ALAWADI FM, KA GUMAA. Studies on the Activity of Individual Plants of an Antidiabetic Plant Mixture, ActaDiabetologica Latina, Vol. 24, No. 1, 1987, pp. 37-41. Available from: ht tp://dx.doi.org/10.1007/BF02732051

[5] BAWANKAR R, DEEPTI, SINGH, SUBASHKUM AR, VIVEKANANDHAN, BABU. 2013. Evaluation of Bioactive Potential of an Aloe vera Sterol Extract. Phytother. Res. 27: 864868. DOI: 10.1002/ptr.4827

[6] CENTERS FOR DISEASE CONTROL AND PREVENTION (2015). Available from: http://www. cdc.gov/media/presskits/aahd/diabetes.pdf

[7] CHARISHMA Y, THIRUVENGADARAJAN. 2013. A review on identification strategy of phytoco nstituents present in herbal plants. Int. J. Res. Pharm. Sci., 4(2), 123-140

[8] Diabetes Medication Side Effect, Available online at Diabetes.co.uk.

[9] DK PATEL, K PATEL, V TAHILYANI. 2012. Barba loin: A concise report of its pharmacological and analytical aspects. Asian Pac J Trop Bio med 2012; 2(10): 835-838, doi:10.1016/S 2221-1691(12)60239-1

[10] DNYANESHWAR AM. 2008. Process of manufac turing clear juice from the leaves of the Aloe vera plant [cited December 2015]. Available from : http://www.google.com.ph/patents/US7 329421

[11] DURAISAMYGUMATHI, MANOKARANKALAISE LVI, CHANDRASEKAR UMA. In Vitro alpha- amylase and -glucosidase inhibitory effects of ethanolic extract of Evolvolusalsinoides(L.)

[12] GHANNAM, M KINGSTON, IA AL-MESHAAL, M TARIQ, NS PARMAN, WOODHOUSE. The Antidiabetic Activity of Aloes: Preliminary Clin ical and Experimental Observations, Hormne Research, Vol. 24, No. 4, 1986, pp. 286-294. http://dx.doi.org/10.1159/000 180569

[13] INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) Aloe Vera. Available from: http://monographs.iarc.fr/ENG/Monographs/ol108/ mono108-01.pdf

[14] JAWADE NR, S CHATTOPADHYAY. 2011.Optimiz ation of Batch Extraction Parameters for Aloin from Aloe Vera Gel.

[15] KIM K ET AL. 2009. temic and hypolipidemic effects of processed Aloe vera gel in a mouse model of non-insulin dependent diabetes mellitus. Phytomedicine doi:10.1016/j.phymed.200 9.02.014 bibitemb.p KWON YI,

APOSTOLIDIS E, SHETTY K. Evaluation of pepper (Capsicum annuum) for mana gement of diabetes / hypertension. Journal of Food Biochemistry. 2007; 31(3): 370385.

[16] MANOHAR V, TALPUR NA, ECHARD BW, LIEBERMAN S, PREUSS HG. Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. Diabetes, Obesity Metabolism. 200 2;4(1):4348. [PubMed]

[17] MATSUDA H, MORIKAWA T, YOSHIKAWA M. Antidiabetogenic constituents from several natural medicines. Pure and Applied Chemist ry 2002;74(7):13011308.

[18] MATSUI T, OGUNWANDE IA, ABESUNDARA KJM, MATSUMOTO K. Anti-hyperglycemic potential of natural products. Mini-Reviews in Medicinal Chemistry. 2006; 6(3):349356. [PubMed]

[19] MINDANAOBOB. 2015, March 27. Insulin in the Philippines [retrieved 12 April 2016) Available from: http://liveinthephilippines.com/content/in sulin-in-the-philippines/

[20] Pharmacelsus Contract Research Organisation, Available online www.pharmacelsus .de/ic50

[21] SACC D, BOGONI P, AND PROCIDA G. 2001. Aloe exudate: characterization by reversed phase HPLC and headspace GC-MS. J Agric Food Chem, 49(10):452630.doi:10.1021/jf0 10179c PMID:11599983

[22] Spectrophotometry: Absorption Measurement and their Application to Quantitative Analysis. 20 05, June. ¡employees.oneonta.edu/kotzjc/LA B/Spec_intro.pdf¿ April 13 2016

[23] SURJUSHE A, R VASANI, DG SAPLE. (2008). Aloe Vera: a short review. Indian Journal of Dermatology. 2008;53(4):163166. Available from https://www.ncbi.nlm.nih.gov/pmc/article /PMC2763764/

[24] TANAKA, ET. AL. Identification of Five Phytost erols from Aloe vera Gel as Antidiabetic Com pounds, Biological and Pharmaceutical Bulle tin, Vol. 29, No. 7, 2006, pp.1418- 1422. Http: //dx.doi.org/10.1248/bpb.29.1418

[25] TARKASE KN, DANVE A V. 2015. Formulation evaluation and in-vitro drug release charac teristics of aloe vera herbal suppositories. Der Pharmacia Lettre, 2015, 7 (2):310-316.

[26] YAGI, Y SATO, Y MIWA, A KABBASH, S. MOUS TAFA, K SHIMOMURA A. EL - BASSUONY, Ribosomal DNA Sequence Analysis of Diff erent Geographically Distributed Aloe vera Plants: Comparison with Clonally Regenera ted Plants, Saudi Pharmaceutical Journal, Vol. 14, No. 3-4, 2006, pp. 208-211.

[27] Wisegeek Clear Answers for Common Questions, Available online at http://www.wisegeek.org/w hat-is-resin.htm

[28] Worthington Biochemical Corporation. 2015. Alph a-Amylase, Assay. Available from: http://www.worthington-biochem.com/AA/default.html

[29] YONGCHAIYUDHA, RUNGPITARANGSI, BUNY APRAPHATSARA, CHOKECHAIJAROENPO RN. 1996. Antidiabetic activity of Aloe vera L. juice. I. Clinical trial in new cases of diabetes mellitus. Phytomedicine Vol. 3, pp. 241-243,