

Alpha-Glucosidase Inhibitory Activity and Fractionation of Bioactive Compounds from bark Extracts of Sibucaco (*Caesalpinia sappan* L.) In the Philippines

Ea Kristine Clarisse B. Tulin, Ma Theresa P. Loreto, Edgardo E. Tulin

ABSTRACT

Diabetes is a chronic disease that affects millions of people around the world. Although conventional medicine is available, it is usually accompanied by serious side effects such as gastrointestinal problem. Thus, the search for alternative medicine from natural sources is of primary importance. **Context:** The study is concerned with *Caesalpinia sappan* L. found in the vicinity of Baybay City, Leyte Philippines. Furthermore, it deals with ethanolic and aqueous extracts of the bark only. It was conducted to determine the alpha-glucosidase inhibitory activity of *C. sappan* to provide evidence to support its efficacy as a folkloric medicinal plant. **Aims:** This work aimed to evaluate the alpha-glucosidase inhibitory activity of aqueous and ethanolic bark extracts of Sibucaco (*Caesalpinia sappan* L.) and to partially fractionate its bioactive compounds. **Settings and Design:** Extracts were prepared in increasing concentration to get IC_{50} values. Samples were analysed in triplicate both for extracts and fractions collected from chromatography. **Methods and Material:** *Caesalpinia sappan* L. bark samples were collected from the vicinity of Visayas State University. Plant materials were dried, weighed and stored. Aqueous and ethanolic bark extracts were prepared using water and 20% ethanol. The total phenolic content of the extracts were determined and the α -glucosidase inhibitory activity of crude bark and aqueous extracts were measured using the method of Kim *et al.*, 2011. Partial fractionation of bioactive compounds was done successively using gradient elution. The α -glucosidase inhibitory activity of fractions were then determined and Rf values of active compounds were measured using thin layer chromatography (TLC). **Results:** Results revealed that aqueous extract (2000 ppm) and ethanolic extract (2000 ppm) show high α -glucosidase inhibitory activity of 100% and 59% respectively. IC_{50} values of aqueous and ethanolic extract were found to be 300.52 ± 1.467 ppm and 1560.81 ± 2.582 ppm, respectively. TLC of fractions show 7 peaks for aqueous extract and 6 peaks for ethanolic extract in which compound with Rf value 0.70 had the highest inhibitory activity of 65%. **Conclusions:** Taken together, the high α -glucosidase inhibitory activity of aqueous extracts support the effectiveness of *C. sappan* L. bark decoctions. In addition, results from bioassay-guided fractionation can be used as an effective tool for further isolation and characterization of α -glucosidase inhibitors from *Caesalpinia sappan* L. in the Philippines.

Key words: *Caesalpinia sappan*, α -glucosidase, Type II diabetes, Flavonoids, Fractionation.

Key Messages: *Caesalpinia sappan* L. (Sibucaco/sappanwood) is used in the islands of the Philippines as folkloric treatment to various ailments including diabetes. This study gives evidence to support the efficacy of bark decoctions of this plant and further shows its potential for medical applications.

Ea Kristine Clarisse B. Tulin, Ma Theresa P. Loreto, Edgardo E. Tulin

Department of Biotechnology, Visayas State University, President, Visayas State University, PHILIPPINES.

Correspondence

Ea Kristine Clarisse B. Tulin

President's cottage, Visayas State University, Baybay City, Leyte 6521, PHILIPPINES.

Phone no: 9068090478

E-mail: eakristine8995@gmail.com

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INTRODUCTION

Conventional medicine of diabetes mellitus available in the market today work by inhibiting α -glucosidase and α -amylase enzymes.¹ Plant extracts are known to possess many therapeutic properties including α -glucosidase inhibitors.² Sibucaco (*Caesalpinia sappan* L.) a plant native to the Philippines has been traditionally used by local healers in the Philippines³ and Southeast Asia⁴ to cure various ailments. This study was conducted to determine the α -glucosidase inhibitory activity of *C. sappan* L. bark extracts, compare enzyme inhibitory activity of aqueous and ethanolic extracts of *C. sappan* L. bark and partially purify and test bioactive compounds responsible for α -glucosidase inhibition.

Subjects and Methods

Chemicals

α -glucosidase (*Saccharomyces cerevisiae*), 4-Nitrophenyl α -D-glucopyranoside (PNPG), Dimethylsulfoxide (DMSO), Standard gallic acid and Acarbose ($\geq 95\%$) were purchased from Sigma-Aldrich, Singapore. Anhydrous sodium carbonate (Na_2CO_3) and Folin-Ciocalteu's phenol reagent were purchased from Merck Millipore, Philippines.

Collection of bark samples

Bark samples of Sibucaco (*Caesalpinia sappan* L.) were collected from verified samples within the area of the Visayas State University, Baybay City, Leyte, Philippines. This was done by scraping portions of the bark

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off the trunk or the branch of the tree. Only the bark part of the tree was used in this experiment.

Preparation of aqueous and ethanolic crude bark extracts

The collected bark samples were washed with distilled water and oven-dried at 40°C for 24 hours. The weight of fresh sample and the weight of dried sample were recorded and percent moisture was determined prior to extraction. Aqueous and ethanolic extracts were prepared using the method of Jyothi *et al.*, 2011.⁵ One hundred grams of dried and ground bark was macerated with 500 mL of solvent, sterile distilled water for aqueous extract and 20% ethanol for ethanolic extract, in a heavy duty homogenizer for 15 minutes. The suspension was filtered through a double layer muslin cloth and then centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered using Whatman no.1 filter paper and the extracts were concentrated using a speedvac concentrator at 40°C and then preserved aseptically at 4°C until further use. Prior to the assay, the extracts were filtered using a 20 micron filter to remove possible microbial contaminants.

Total phenolic content of plant extracts

Crude extracts were reconstituted with dimethylsulfoxide (DMSO) and total phenolic concentration of sample was determined using the method by Singleton *et al.* (1999)⁶ with some modifications. Standard gallic acid solutions of 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm concentrations were prepared. About 0.5 mL of the sample was added with 2.5 mL Folin-Ciocalteu solution (1:10 dilution with water) and 2.5 mL Na₂CO₃ (75 g/L). The mixture was allowed to react for 30 min at 25°C. Absorbance was read at 765 nm using UV-Vis spectrophotometer and concentration of extract were determined using the standard calibration curve.

Evaluation of alpha-glucosidase inhibitory activity

The method used to evaluate the inhibitory activity of *C.sappan* was based on Kim *et al.* (2011).⁷ Concentrated aqueous and ethanolic extracts were reconstituted with distilled water and DMSO, respectively, at 100 ppm, 500 ppm, 1000 ppm and 2000 ppm concentrations. Extracts (50 µL, at different concentrations) were incubated with α-glucosidase (50 µL, 0.5 Units/mL) and 0.2 M potassium phosphate buffer (50 µL, pH 6.8) at 37°C in a waterbath for 15 min. Then, 100 µL of 3 mM p-nitrophenyl glucopyranoside (PNPG) was added as substrate. The reaction was incubated again for ten minutes and then stopped by the addition of 750 µL of 0.1 M Na₂CO₃. The absorption of 4-nitrophenol, a product after reaction, was measured at 405 nm using a UV-Vis spectrophotometer. Reaction mixture without the sample served as negative control and reaction mixture without the substrate served as blank. This experiment was performed in triplicate. Acarbose was used as positive control.

The percentage inhibition of α-glucosidase was computed as follows:

$$\text{Inhibition rate (\%)} = \{1 - (\text{Abs S} - \text{Abs B}) / \text{Abs C}\} \times 100$$

where: Abs S = absorbance of the experimental sample,

Abs B = absorbance of the blank

Abs C = absorbance of the negative control

Chromatographic separation of bioactive compounds using column chromatography (Shiny *et al.*, 2013)⁸

Crude extracts were evaporated in vacuo using a speedvac and the concentrate (pellet) was resuspended in absolute ethanol, at 1:1 ratio. Glass chromatographic column (15 cm long and 1.5 cm diameter) was prepared using silica gel (packed 9.0 cm long) equilibrated with three column volumes of hexane. Gradient elution with increasing polarity was done and fifteen fractions of one mL volumes each were collected. Table 1 shows the solvent systems used and the corresponding fraction num-

ber. Fractions were further tested for its α-glucosidase inhibitory activities using the previously described inhibition assay.

Thin layer chromatography (Shiny *et al.*, 2013)

For thin layer chromatography, fractions were evaporated in vacuo using a speedvac and the resulting concentrate was resuspended in ethanol. Silica gel 60 F 254 plate (20 cm x 20 cm) was used as the stationary phase and hexane and ethanol in a 9:1 ratio was used as the mobile phase. The samples that were used are the solvent alone and fractions collected. The samples (2µL) were spotted on TLC plates at an equal distance of one cm. Thereafter, the plates were viewed in UV light at 245 nm and spots were properly documented. Rf values were calculated after viewing the chromatogram using the formula

$$Rf = \frac{\text{Distance (cm) from the origin to the center of substance spot}}{\text{Distance (cm) from origin to solvent front}}$$

RESULTS

Moisture content of plant sample

After oven-drying at 40°C for 26 hours the bark extract had a moisture content of 36.80%. Moisture content analysis of a plant sample is important since it indicates the quality of material. In addition, when extracting phytochemicals from plant sample it is necessary to use dried samples as to inhibit the degradation of compounds and to aid in long term storage or preservation of sample.⁹

Total phenolic content

The phenolic content of aqueous and ethanolic extract was determined to have a baseline concentration of phenolic compounds in the sample. Phenolic content was used as basis of varying concentration since most α-glucosidase inhibitors derived from plant extracts are phenolic in nature.^{10,11,12} Moreover, flavonoids have also been known to possess hypoglycemic activity.¹³

Alpha-glucosidase inhibitory activity of aqueous and ethanolic extracts

Table 3 shows the α-glucosidase inhibitory activity of ethanolic and aqueous extracts at phenolic concentrations of 100 ppm, 500 ppm, 1000 ppm and 2000 ppm. The IC₅₀ value for ethanolic extracts is 1569.81 ± 2.582 ppm and for aqueous extracts is 300.52 ± 1.467 ppm.

extracts at different concentrations

Figure 1 shows the graphical representation of the alpha-glucosidase inhibitory activity of aqueous and ethanolic extracts at different concentrations. Furthermore, it shows the dose-dependent response of the extracts Table 4 illustrates the α-glucosidase inhibitory activity of the positive control Acarbose at different concentrations. The evaluation of alpha-glucosidase inhibitory activity of acarbose was done to assess the validity of assay used. The concentration of Acarbose needed to inhibit α-glucosidase activity by half is 0.02043 ppm.

Bio-assay guided fractionation of aqueous extracts

To fractionate bioactive compounds, column chromatography was done using 15 solvent systems and 15 fractions from aqueous extract were collected. Figure 2 shows the thin layer chromatogram (TLC) of the collected aqueous fractions while Table 5 shows the corresponding fraction number, inhibitory activity, Rf value and solvent system used in chromatographic separation of aqueous extracts.

Table 1: List of solvent systems used for column chromatography

Fractions 1-5	Fractions 6-10	Fractions 11-15
Hexane: Ethyl acetate 9:1	Ethyl acetate: Ethanol 9:1	Ethanol:Methanol 9:1
Hexane: Ethyl acetate 7:3	Ethyl acetate: Ethanol 7:3	Ethanol:Methanol 7:3
Hexane: Ethyl acetate 5:5	Ethyl acetate: Ethanol 5:5	Ethanol:Methanol 5:5
Hexane: Ethyl acetate 3:7	Ethyl acetate: Ethanol 3:7	Ethanol:Methanol 3:7
Hexane: Ethyl acetate 1:9	Ethyl acetate: Ethanol 1:9	Ethanol:Methanol 1:9

Table 2. Total phenolic content of aqueous and ethanolic extracts

Extract	Concentration (mg/mL)	Phenolic content (GAE/mL)
Aqueous	42.50	1826 ppm
Ethanolic	63.925	6516 ppm

Table 3: Alpha-glucosidase inhibitory activity of aqueous and ethanolic extracts at different concentrations

Concentration	% Inhibitory activity	
	Ethanolic extracts	Aqueous extracts
100 ppm	19.55 ± 3.934	43.65 ± 1.067
500 ppm	28.16 ± 1.407	56.14 ± 2.724
1000 ppm	37.22 ± 0.644	88.68 ± 0.665
2000 ppm	59.72 ± 4.432	100.00 ± 0.700
IC ₅₀ values	1560.81 ± 2.582 ppm	300.52 ± 1.467 ppm

Table 4: Alpha-glucosidase inhibitory activity of Acarbose, positive control

Concentration (µg/mL)	Inhibitory activity (%)
0.01	29.97 ± 4.929
0.02	66.83 ± 1.932
0.04	78.93 ± 0.089
0.06	80.24 ± 2.334
0.08	84.37 ± 0.385
0.10	94.14 ± 0.194
IC50 (Acarbose)	0.02 ppm ± 1.644

Bio-assay guided fractionation of ethanolic extracts

Column chromatography yielded 15 fractions from ethanolic extract as well using 15 solvent systems. Table 6 shows the corresponding fraction number, inhibitory activity, Rf value and solvent system used in chromatographic separation. Figure 3 shows the thin layer chromatogram (TLC) of the collected ethanolic fractions.

DISCUSSION

Total phenolic content

Table 2 shows that the phenolic content of ethanolic extract is 3.5 times higher (6516 ppm) than the aqueous extract (1826 ppm). Ethanol has a polar and non-polar end which makes it an ideal solvent for the extraction of secondary phytoconstituents such as phenolics since it can extract both polar and non-polar components. The solubility of phenolics in water is also lower compared to ethanol which contributes to more phenolics being extracted by ethanol compared to water.

Alpha-glucosidase inhibitory activity of aqueous and ethanolic extracts

Results revealed a dose-dependent response because increasing the concentration of ethanolic and aqueous extracts increased the inhibitory activity to 59.72% and 100%, respectively. Likewise, in terms of half maximal inhibitory concentration (IC₅₀) which is the concentration needed for the sample to inhibit α-glucosidase by 50%, Table 3 shows that ethanolic extract has a higher IC₅₀ value of 1560.81 ppm compared to aqueous extract with IC₅₀ value of 300.52 ppm. This suggests that the

Table 5: Inhibitory activity of aqueous fractions

Fraction number	Solvent system	% Inhibitory activity	Rf value
1	Hexane: Ethyl acetate 9:1	-2.058	0.68
6	Ethyl acetate: Ethanol 9:1	12.954	0.70
9	Ethyl acetate: Ethanol 3:7	38.136	0.70
10	Ethyl acetate: Ethanol 1:9	51.695	0.70
11	Ethanol: Methanol 9:1	65.012	0.71
12	Ethanol: Methanol 7:3	19.492	0.63
13	Ethanol: Methanol 5:5	-79.056	1 0.18 2 0.58 3 0.67 4 0.85 5 0.95
14	Ethanol: Methanol 3:7	-63.317	1 0.18 2 0.67 3 0.85 4 0.95
16	Ethanol: Methanol 1:9	-157.022	1 0.65 2 0.85 3 0.95

Table 6: Alpha-glucosidase inhibitory activity of ethanolic fractions

Fraction number	Solvent system	% Inhibitory activity	Rf value
5	Hexane:Ethyl acetate 1:9	-12.91	1 0.57 2 0.63 3 0.79 4 0.94
6	Ethyl acetate: Ethanol 9:1	-22.55	1 0.58 2 0.65 3 0.79 4 0.93
8	Ethyl acetate: Ethanol 5:5	58.29	0.70
9	Ethyl acetate: Ethanol 3:7	65.39	0.70
10	Ethyl acetate: Ethanol 1:9	58.01	0.71
11	Ethyl acetate: Ethanol 9:1	58.01	0.70
12	Ethanol: Methanol 7:3	62.55	0.71
13	Ethanol: Methanol 5:5	-30.50	0.65
14	Ethanol: Methanol 3:7	-27.36	0.67

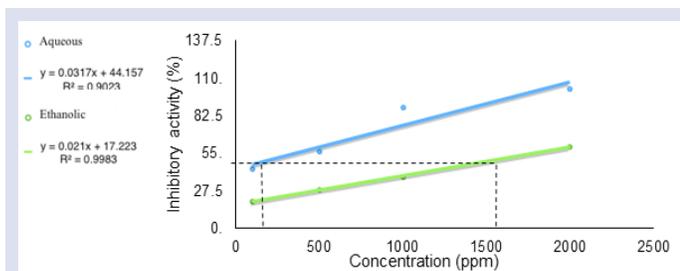


Figure 1: Alpha-glucosidase inhibitory curve of aqueous and ethanolic fractions.

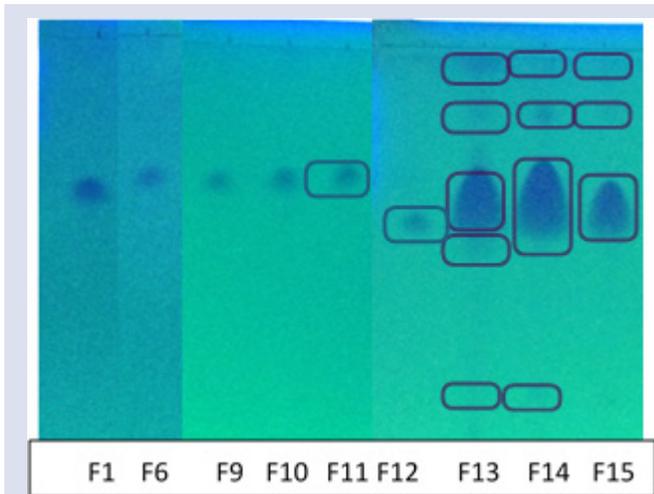


Figure 2: Thin layer chromatogram of aqueous fractions.

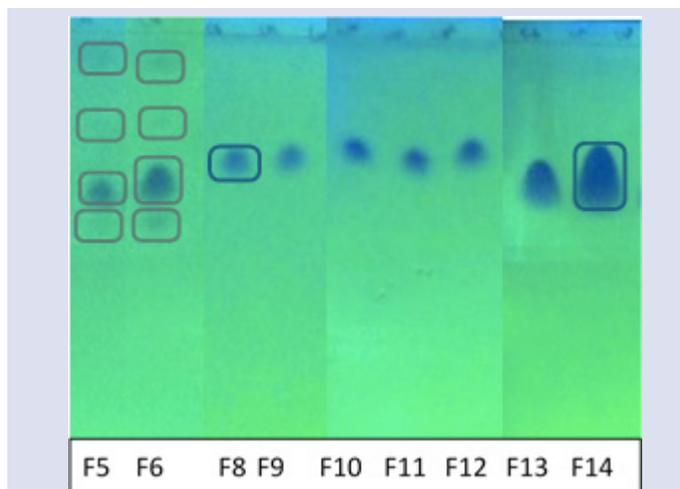


Figure 3: Thin Layer Chromatogram of ethanolic fractions.

inhibitory activity of aqueous extract is higher compared to the inhibitory activity of ethanolic extracts.

Figure 1 shows the linear relationship between concentration and α -glucosidase inhibitory activity of aqueous and ethanolic bark extracts. The increasing inhibitory activity (%) directly related to the increase in concentration of phenolic compounds implies that there is a dose-dependent response of the extract and that the phenolic components in the extract are highly responsible for its α -glucosidase inhibitory activity. This narrows down the possible class of compound which the α -glucosidase inhibitor belongs. In addition, figure 2 also indicates that aqueous extracts generally have higher α -glucosidase activity compared to ethanolic extracts. This is consistent with the half maximal inhibitory

activity values. This further suggests the polarity of inhibitor compound present in the extract.

Bio-assay guided fractionation of aqueous extracts

Table 5 shows the increasing α -glucosidase inhibitory activity of fractions 1-11, wherein fraction 11 has the highest inhibitory activity of 65.01%. In addition, TLC of these fractions shows the presence of clear single spots with Rf value of 0.70. This suggests the presence of α -glucosidase inhibitors separated from other compounds in the extract. Fraction 12 showed a decrease in α -glucosidase inhibitory activity while fractions 13, 14 and 15 show a negative α -glucosidase inhibitory activity. This indicates that these fractions no longer contained α -glucosidase inhibitors. TLC of fraction 12 shows a slightly lower spot compared to previous fractions with an Rf value of 0.63 while fractions 13-15 show multiple spots with Rf values of 0.18, 0.58, 0.67, 0.85 and 0.95. Since these fractions were collected in the later part of elution, they may already contain wash residues, pigments and other flow-through components which are not α -glucosidase inhibitors but larger molecules that might mask the activity of the α -glucosidase inhibitor activity. Furthermore, in this study, only inhibitory activities of clear fractions were considered, since turbid fractions collected at the later part of fractionation contained background noise that are most likely pigments and wash residues. Multiple possible bioactive and non-bioactive components were identified in the extract using results of bioassay guided fractionation with Rf values of 0.18, 0.58, 0.63, 0.67, 0.70, 0.85 and 0.95.

Bio-assay guided fractionation of ethanolic extracts

Fractions one to five collected in the early stages of separation showed low inhibitory activity while fraction 6 showed negative inhibitory activity. TLC of these fractions shows a combination of compounds with Rf values of about 0.57, 0.63, 0.79 and 0.94. The negative and low inhibitory activities of these fractions might be attributed to the presence of several compounds in the fraction as observed in the number of spots detected. Ethanolic extract contains more non-polar components compared to aqueous extracts, and these non-polar components might have masked the activity of α -glucosidase inhibitors. This is evident in the low and negative inhibitory activities of fractions one to six collected from non-polar solvent systems. Fractions 8 to 12, collected from mid to highly polar solvent systems showed consistently high inhibitory activities with maximum inhibitory activity of 65.39%. In addition, TLC of these fractions show single spots with similar diameter and appearance and with an average Rf value of 0.70. Since these fractions possess high α -glucosidase inhibitory activity they have high potential as α -glucosidase inhibitors. Fractions 13 and 14 show a decrease in inhibitory activity reaching highly negative values indicating that these fractions do not contain α -glucosidase inhibitors. Similar to purification done with aqueous extracts, fractions collected that were turbid were not considered. TLC of these fractions show a tailing spot with average Rf value of 0.65. Tailing spot implies poor separation of components which may need alternative solvent system for separation.

Implications

The search for sources of alpha-glucosidase inhibitors from indigenous plants is a strategy employed in this study to provide an alternative treatment for diabetes mellitus. It was shown from the results presented that the aqueous extract from the barks of *Sibucao* possessed high inhibitory activity suggesting that water decoctions from the bark maybe enough to extract its medicinal property. This result is also consistent with the IC_{50} value which measures the activity of the enzyme.

The Rf value of 0.70 for the isolated fraction also indicates the property of the inhibitor that may provide us with information on its potential iden-

tity and biological properties. Furthermore, the result of this separation is also useful for future characterization of the inhibitory compound.

Taken together, this study has shown evidence of the potential of Sibucao as a natural source of α -glucosidase inhibitor for treatment of diabetes and offers an explanation of why local “herbal healers” recommend this plant to remedy the said ailment.

CONCLUSION

Based on the results obtained, aqueous and ethanolic crude bark extracts of *Caesalpinia sappan* L. possess promising α -glucosidase inhibitory activity. At 2000 ppm concentration, aqueous extracts show 100.00% and ethanolic extracts show 59.72% inhibition. Generally, aqueous extracts have higher enzyme inhibition activity compared to ethanolic extracts. In terms of half maximal inhibitory activity (IC_{50}), aqueous extracts have IC_{50} of 300.52 ppm and ethanolic extracts have IC_{50} of 1560.81 ppm. Partial fractionation and testing of bioactive compounds revealed the appearance of 7 peaks for aqueous extracts with Rf values of 0.18, 0.58, 0.63, 0.67, 0.70, 0.85 and 0.95 and 6 peaks for ethanolic extracts with Rf values of 0.57, 0.63, 0.67, 0.70, 0.79 and 0.93. Both aqueous and ethanolic extracts contain compound with Rf value of 0.70, this compound shows positive inhibitory activity of 65%. Results from this study can be used as a reference tool for future isolation and characterization of α -glucosidase inhibitors.

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CONFLICTING INTEREST

This study has no conflict of interest.

ABBREVIATIONS USED

TLC: Thin Layer Chromatography; IC_{50} : Half Maximal Inhibitory Activity; PNPg: 4-Nitrophenyl α -D-glucopyranoside; DMSO: Dimethylsulf-

oxide; Abs: Absorbance; α -glucosidase: Alpha-glucosidase; Rf values: Retention Factor values.

REFERENCES

- Mcculloch D, Nathan D, Mulder J. Patient information: Diabetes mellitus type 2: Treatment (Beyond the Basics). Up to date [Internet] Available from: <http://www.uptodate.com/contents/diabetes-mellitus-type-2-treatment-beyond-the-basics>.
- Lee SS, Lin HC, Chen CK. Acylated flavonol monorhamnosides, α -glucosidase inhibitors, from *Machilus philippinensis*. *Phytochem*. 2008;69(12):2347-53. <https://doi.org/10.1016/j.phytochem.2008.06.006>; PMID:18639907.
- Del fierro RS, Nolasco FA. An exploration of the ethno-medicinal practices among traditional healers in southwest cebu, philippines. *APRN Journal of Science and Technology*. 2013;3:1184-86.
- Wake up Philippines [Internet]. Erineus. 2009. Health benefits of Sappan wood. Alternative medicine [cited: May 19, 2014]. Available from: <https://wakeuphiils.wordpress.com/tag/sibucao/>
- Jyothi KS, Hemalatha P, Challa S. Evaluation of α -amylase inhibitory potential of three medicinally important traditional wild food plants of India. *International journal of green pharmacy*. 2011;5(2):95-9. <https://doi.org/10.4103/0973-8258.85158>.
- Singleton VL, Orthofer R, Lamuela-raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. *Methods Enzymol*. 1999;299:152-78. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- Kim J, Yang J, Kim M. Alpha glucosidase inhibitory effect, anti-microbial activity and UPLC analysis of *Rhus verniciflua* under various extract conditions. *Journal of Medicinal Plant Research*. 2011;5(5):778-83.
- Shiny C, Saxena A, Gupta S. Phytochemical investigation of the insulin plant “*Costus pictus*” D. Don. *International J Pharm Biomed Res*. 2013;4:97-104.
- Fao.org [Internet]. _ [cited: March 11, 2016] Available from: <http://www.fao.org/docrep/X5036E/x5036E0p.htm>.
- Kumar S, Narwal S, Prakash O. α -glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Review* 2011;5(9):19-29. <https://doi.org/10.4103/0973-7847.79096>; [https://doi.org/10.1016/S0975-3575\(11\)80021-0](https://doi.org/10.1016/S0975-3575(11)80021-0).
- Hanamura T, Hagiwara T, Kawagishi H. Structural and functional characterization of polyphenols isolated from acerola (*Malpighia emarginata* DC.) fruit. *Biosci Biotechnol Biochem* 2005;69:280-6. <https://doi.org/10.1271/bbb.69.280>; PMID:15725651.
- Thilagam E, Parimaladevi B, Kumarappan C, Mandal SC. α -Glucosidase and α -amylase inhibitory activity of *Senna surattensis*. *J Acupunct Meridian Stud*. 2013;6:24-30. <https://doi.org/10.1016/j.jams.2012.10.005>; PMID:23433052.
- Song Y, Manson JE, Buring JE, Sesso HD, Liu S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *J Am Coll Nutr* 2005;24(5):376-84. <https://doi.org/10.1080/07315724.2005.10719488>; PMID:16192263.

SUMMARY

- Alpha-glucosidase inhibitory activity of aqueous extracts were determined.
- IC_{50} value = 300.52 \pm 1.467 ppm
- Alpha-glucosidase inhibitory activity of ethanolic extracts and IC_{50} were determined.
- IC_{50} value = 1560.81 \pm 2.582 ppm
- Partial fractionation was done using column chromatography to isolate bioactive compounds. 15 solvent systems were used, collecting fifteen 1mL fractions from each extract.
- Bioactivity of each fraction was determined by measuring the α -glucosidase inhibitory of each fraction.
- Active compounds were analyzed using Thin Layer Chromatography and Rf values were measured.
- Compound with Rf value of 0.70 had the highest α -glucosidase inhibitory activity of 65% for aqueous and ethanolic extracts.

ABOUT AUTHORS



Ea Kristine Clarisse Tulin: Is an Instructor at the Department of Biotechnology of the Visayas State University-Baybay City, Leyte, Philippines. She graduated Bachelor of Science in Chemistry at the same university. For her undergraduate thesis she worked on a diabetes related study which involved the evaluation of the alpha-glucosidase inhibitory activity of natural products, specifically *Caesalpinia sappan* L. bark extracts, commonly known as Sibucao.

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