In-Vitro α-Amylase and α-Glucosidase Inhibitory Activities of Flavonoids from *Eleusine indica* (Linn.) Gaertn. Leaf Extract

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Abstract

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.The present study was designed to investigate the α-amylase and α-glucosidase inhibitory activity of Eleusine indica leaves. Preliminary α-amylase inhibitory activity of *E. indica* showed IC50= 118.3 µg/mL against α-amylase from *Aspergillus oryzae* and IC50= 119.1 µg/mL against α-amylase from porcine pancreas. The standard drug acarbose showed an IC50= 0.00001818 mg/mL. The plant extract was subjected to column chromatography and obtained 35 fractions and was pooled based on TLC profiling. The 5 pooled fractions were tested to acquire the total phenolic content. Pooled fractions 1 and 2 have the highest TPC and total flavonoid content of these fractions was also determined. The two pooled fractions were subjected to α-amylase (porcine pancreas) and α-glucosidase *(Saccharomyces cerevisiae)* inhibitory assay. The plant extract (IC50=141.4 µg/mL) showed minimal inhibitory activity than the standard drug acarbose (IC50=0.00001818 mg/mL) against α-amylase. The plant extract (IC50=101.6 µg/ml) also showed minimal inhibitory activity than the standard drug acarbose (IC50 =of 122.9 µg/ml) against α-glucosidase.

Keywords: *Eleusine indica , Diabetes, Flavonoids, α- Amylase, α-Glucosidase*

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder that persistently occurs either when the pancreas becomes incapable of producing enough insulin or when the body still produces insulin but cannot efficiently use it to compensate the need of the body (WHO, 2017). It is associated with impaired metabolism of glucose leading to microvascular and macrovascular changes in due course causing complications that are difficult to manage. Diabetes has significant impact on Third World countries, especially the Philippines. Asia is said to have the largest increase in the number of diabetes patients by 2025. This increase in the burden of chronic diseases in Asia will have a significant impact on the acute and chronic healthcare systems of the respective countries (Tan, 2015). Type II DM is, by far the most widespread type. It is characterized by impaired metabolism of lipid, carbohydrates, and lipid with defects in the secretion of insulin, roughly contributing to the majority of insulin resistance (Etxeberria, de la Garza, Campión, Martínez, & Milagro, 2012)

Reports indicate that hyperglycemia, manifested as elevated sugar in the blood is one of the effects of unrestrained diabetes. It may lead to severe impairment of the body's system, particularly in the nerves and vessels of the blood over the time (Gaikwad, Krishna Mohan, & Sandhya Rani, 2014). Hyperglycemia is known to be related to the increase prevalence of microvascular complications in type II DM (Shim et al., 2003). According to some studies, there are many ways to control diabetes using pharmacological treatments that targets mechanisms of action (Thilagam, Parimaladevi, Kumarappan, & Chandra Mandal, 2013). One of these is to lessen post-prandial elevation of blood sugar by impeding with its absorption through preventing the breakdown of carbohydrates in the digestive tract. Such enzymes are called α-amylase and α-glucosidase (Ali, Houghton, & Soumyanath, 2006).

A number of anti-diabetic drugs, like miglitol, voglibose, acarbose, nojirimycin, sitagliptin, and 1-deoxynojirimycin, aim different glucosidases in the digestive tract, particularly maltase, sucrase, and α-amylase, lowering glucose levels after ingestion of foods. However, these drugs lack specificity thereby producing several undesirable effects in the gastrointestinal tract such as flatulence, diarrhea, and abdominal cramping that limits its use although proven safe and tolerable (Etxeberria et al., 2012).

Several studies showed that bioactive molecules and biologically active natural substances have recently become a major source of medicinal plants and food and have been recognized as effective against disease and complications (Benalla, Bellahcen, & Bnouham, 2010). One of the phytochemicals present in some plants are flavonoids which are

beneficial group of hypoglycemic potentially occurring compounds. They can enhance altered glucose and diabetic oxidative metabolisms. The hypoglycemic effect of certain herbal extracts was confirmed in type 2 diabetes mellitus models for humans and animals (Gaikwad et al., 2014).

Figure 1. Eleusine indica (Paragis) plant from Ibaan, Batangas

Eleusine indica (Fig.1) commonly known as "Goosegrass" and locally known as "paragis", generally considered an adventitious species, is native in the tropics and subtropical regions. It is a native plant in tropical and subtropical regions, such as the Philippines. Phytochemical analysis on *E.indica* proves that its leaves contains alkaloids, terpenes, flavonoids, tannins, anthraquinones, saponins and cardiac glycoside (Okokon, Odomena, Effiong, Obot, & Udobang, 2010). Several studies also showed that this plant exhibit several pharmacologic activities, such as anti-plasmodial (Okokon et al., 2010), antipyretic (Pattanayak & Maity, 2017), antihypertensive (Tutor & Chichioco-hernandez, 2018), antiurolithiatic (Opoku Amoah, 2017), antioxidant, antibacterial and cytotoxic properties (Abdul et al., 2011).

At present, prevalence of diabetes is rising rapidly. For this reason, researchers are finding alternative plant based agents with lesser side effects. The present study aimed to investigate the antidiabetic potential of *E. inidica* leaf extract by α-amylase and α-glucosidase inhibition. This study aimed to determine the fractions with the highest total phenolic and flavonoid content and to determine the anti-diabetic property of *E. indica* leaf extract by inhibition of α-amylase and α-glucosidase in comparison with Acarbose using α-glucosidase and α-amylase inhibition assay. This study is limited to the determination of antidiabetic activity of the leaves of *E. indica.* The purification, isolation and structure elucidation of the active antidiabetic constituent was excluded in this study.

MATERIALS AND METHODS

Materials and Reagents Procured

Methanol TG, Petroleum ether, Chloroform, Ethyl acetate was procured from RTC Laboratories. α-amylase and α-glucosidase enzyme, PNPG was procured from Sigma Aldrich.

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Plant collection and authentication

Fresh leaves of *E. indica* was collected from Brgy. Sabang Ibaan, Batangas and was authenticated at University of Santo Tomas, Sampaloc, Manila.

Preparation of plant

Leaves was collected and air-dried for two weeks in the laboratory condition for easy powdering. The dried leaves was grind into fine powder and then weighed (Temidayo, 2013).

Extraction of plant

For three days, powdered leaves was macerated with 70 % methanol and filtered. The filtrate was concentrated using thermostatic water bath at 80°C. To remove excess fats and oils, the methanol crude material was defatted with petroleum ether (Bajpai, Majumder, & Park, 2016). It was allowed to dry at room temperature (Okwo, 2014). The concentrated extract was sujected to preliminary α-amylase inhibition assay.

Column Chromatography

The column chromatography will be done using the procedure with modification of Sambandam, Thiyagarajan, Ayyaswamy, & Raman, (2016). The concentrated extract (5 g) was subjected to column chromatography with Silica Gel (Sigma-Aldrich 100-200 mesh) eluted with 300 mL chloroform chloroform:ethyl acetate in the ratio of , 7.5:2.5, 5:5, and 2.5:7.5,ethyl acetate, ethyl acetate:methanol in the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, methanol , methanol:distilled water in the ratio of 9:1, 8:2, 7:3, 6:4, and 5:5 to obtain 35 fractions. All the collected fractions with the same compounds were pooled based on the results of TLC.

Thin Layer Chromatography

Thin layer chromatography was employed to screen the presence of flavonoids in the exctract. Thin layer plates coated with silica gel were used. Different solvent systems were used such as chloroform: acetic acid: water (50:45:5) and toluene: acetone: chloroform (40:25:35). The plates were dried and sprayed with antimony (III) chloride to detect the presence of flavonoids. visualization was carried out under UV 365 nm.

Observable result for a positive test was intense yellow to orange.

Total Phenolic Content

TPC was measured using the method with modification of Baba & Malik, (2015). In the determination of total phenolic content, Folin- Ciocalteu phenol reagent was used. The standard used for the test is gallic acid. 200 µL of the plant extract was used. 800 µL of Folin–ciocalteu, phenol reagent was then added. After 5 mins, 600 µL of 20% sodium carbonate was added. The mixture was allowed to stand and kept in darkness for 2 hours. Using UV-Vis spectrophotometer the absorbance of the reaction mixtures will be measured against blank at 420 nm wavelength. A blank sample was prepared using the same solution excluding the extract. The following formula was used to determine the amount of total phenol based on gallic acid standard. The pooled extract with the highest phenolic content was tested for the determination of the total flavonoid content and the same pooled fractions were used in α-amylase and α-glucosidase inhibition assay.

Total Flavonoid Content

The TFC was measured by adopting the method with modifications of Chandra et al., (2014). For the determination of total flavonoid content of the sample, aluminum chloride colorimetric method was used. Quercitin (250, 125, 62.5, 31.25 µg/mL) was used as a standard calibration curve for total flavonoid determination. 50 µL of extract will be mixed with 50 µL of 2% aluminum chloride. The solution was incubated in dark room temperature for 60 minutes after mixing. Using a UV-Vis spectrophotometer, the absorbance of the reaction mixtures will be measured against blank at 420 nm wavelength. The concentration of total flavonoid content in the test samples was calculated from the calibration plot and was expressed as mg quercetin equivalent (QE)/g of dried plant. All the determinations was done in duplicate.

α-amylase Inhibition Assay

The activity was measured by applying the literature procedure with modifications (Chakrabarti, Sigh, VN, Vanchhawng, & Thirumurugan, 2014). The substrate was prepared by dissolving 0.5 g starch in 50 ml of 0.1M PBS (phosphate buffer saline, pH 6.9) and was heated for 15 minutes. The reaction mixture, consisting of 20 µl of α-amylase solution (1U) and 100 µl of plant extract (100, 50, 25, 12.5 µg/ml) was incubated at 37°C for 10 mins, followed by the addition of 100 µl of starch solution and was further incubated at 37°C for 30 mins.

20 µl of 1M HCl was added to arrest the reaction. 50 µL of Iodine reagent (5mM I2 and 5mM KI) and 710 µL of PBS was added. The solution was mixed using vortex. The reaction mixture was then transferred to a 96 well microplate The blue complex was analyzed using microplate reader at 630 nm (HumaReader HS). The mixture that contains only the substrate and PBS was served as a blank to correct background absorbance. The mixture that contains only the enzyme, substrate and PBS was served as a control. Acarbose (100,50,25,12.5,6.25 µg/mL) was used as a positive control. All the tests was done in triplicate. Inhibitory activity was calculated using the following formula:

$$\%\, Inhibition\ = \frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control}\ x\ 100$$

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory assay will be done using a modified procedure of (Asghari, Salehi, Farimani, & Ebrahimi, 2015). The reaction mixture consisting of 100 µl of test sample (100, 50, 25, 12.5 µg/ml) and 50 µl α-glucosidase solution (10 U/mL in PBS) was preincubated in microcentrifuge at 37°C. After preincubation, 100 µl of substrate (10 mM p-nitrophenyl α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) was added to the reaction mixture and it was further incubated at 37°C for 30 minutes and it was transferred to 96 well plate. By the addition of 100 µl of 1M Na2CO3 solution, the reaction will be terminated. Then the absorbance will be measured at 405 nm in microplate reader. The positive control used was acarbose (100, 50, 25, 12.5 µg/ml) and the mixture that contains only the substrate and PBS was served as a blank to correct background absorbance. The mixture that contains only the enzyme, substrate and PBS was served as a control. All the experiment was done in duplicate. The percent inhibition was assessed by the following equation:

$$\%\, Inhibition\ = \frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control}\ x\ 100$$

Statistical Analysis

GraphPad 8.0 version was used to determine the IC50 value of the inhibitors.

RESULTS

Preliminary α-amylase Inhibitory activity of the methanolic extract.

The extract was tested for the α-amylase inhibition assay to detect the activity using α-amylase enzyme from porcine pancreas (5 U) and *aspergillus oryzae**.*** Based on the results,

standard acarbose showed an IC50 value of 0.00001818 mg/mL **(Fig 2).** Two different enzymes were used in preliminary screening of α-amylase inhibition activity. One of those was α-amylase from *aspergillus oryzae* which showed an IC50 value of 118.3 µg/mL **(Fig. 3)**. α-amylase from porcine pancreas inhibition showed an IC50 of 119.1 µg/mL **(Fig. 4).**

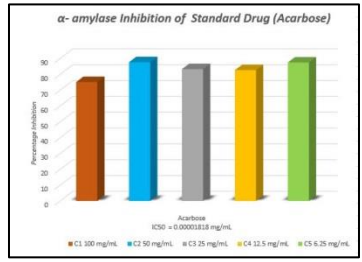

Figure 2. Percentage Inhibition of acarbose against α-amylase

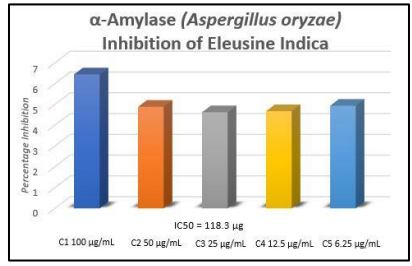

Figure 3. Percentage inhibition of *E. indica* against α-amylase from *Aspergillus oryzae*

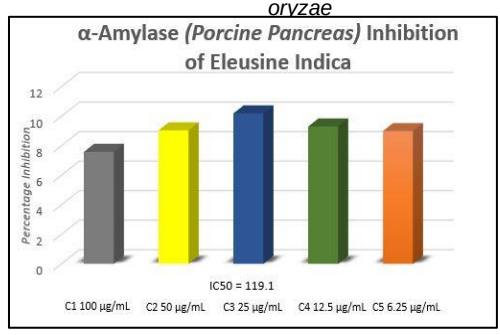

Figure 4. Percentage inhibition of *E. indica* against α-amylase from *Porcine pancreas*

Column Chromatgraphy

The leaf extract (5g) was subjected to column chromatography on silica gel (Sigma-Aldrich 100-200 mesh) eluted with 300 mL chloroform chloroform:ethyl acetate in the ratio of , 7.5:2.5, 5:5, and 2.5:7.5,ethyl acetate, ethyl

acetate:methanol in the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, methanol , methanol:distilled water in the ratio of 9:1, 8:2, 7:3, 6:4, and 5:5 to obtain 35 fractions. All the collected fractions with the same compounds were pooled based on the results of TLC to obtain 5 pooled fractions - Fractions 2-5,6-8, 11-17, 18-29, 30-35 **(Fig. 5).**

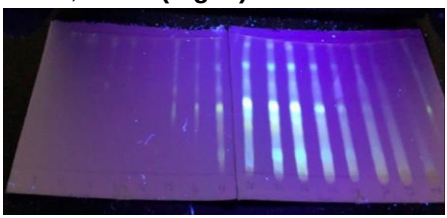

Figure 5. Chromatogram of fractions 2-35 under UV light

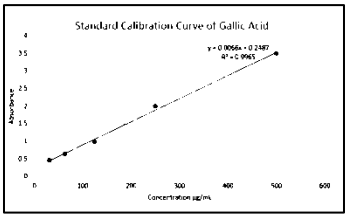

Figure 6. Standard Calibration Curve of Gallic Acid

Total Phenolic Content

All pooled fractions were subjected in quantifying TPC. Pool 1 and 2 have the highest TPC.

Table 1
Gallic Acid Equivalent of E. indica pooled fraction extract

***E. indica* pooled fraction extract**	**Absorbance (mean ± Sd)**	**Gallic acid Equivalent** y = 0.0066x + 0.2487
Pool 1	0.785333 ± 0.2	81.31µg
Pool 2	1.281 ± 0.01	156.41 µg

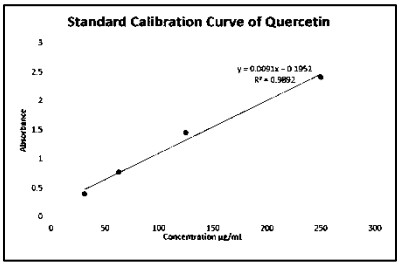

Figure 7. Standard Calibration Curve of Quercetin

Total Flavonoid Content

The pooled fraction extract showed quercetin equivalence of pool 1 (-9.31 µg) and pool 2 (1.30 µg).

α -Amylase inhibitory activity of the pooled fraction extracts

The pooled fraction extracts were subjected to α-amylase inhibition assay. According to the results, standard drug acarbose showed an IC50 value of 0.00001818 mg/mL. Pool 2 which has an IC50 value of 141.4 µg/mL has better inhibition compared to pool 1 which has IC50 value of 153.5 µg/mL **(Fig. 8).**

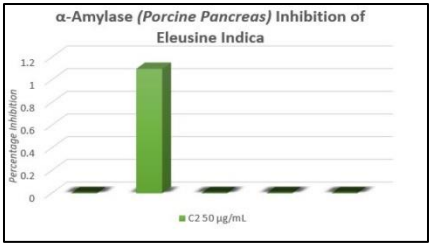

Figure 8. Percentage inhibition of Eleusine indica pooled fractions against α-amylase.

Table 2
Quercetin Equivalent of E. indica pooled fraction extract

***E. indica* pooled fraction extract**	**Absorbance (mean ± Sd)**	**Quercetin Equivalent y = 0.0091x + 0.1952**
Pool 1	0.1105± 0.01	-9.31 µg
Pool 2	0.207±0	1.30 µg

α -Glucosidase Inhibition Activity of the pooled fraction extract

The pooled fraction extract was subjected to α-glucosidase inhibition assay to determine the activity. Standard drug acarbose showed an IC50 value of 122.9 mg/mL **(Fig. 9).** Pool 1 (IC_{50}=101.6 µg/ml) showed better inhibition against alpha glucosidase. (**Fig. 10).**

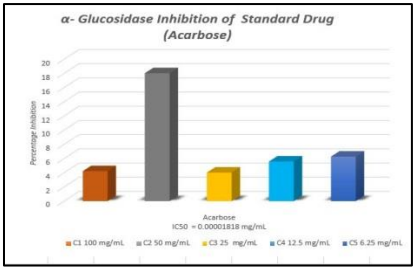

Figure 9. Percentage inhibition of acarbose against α-glucosidase

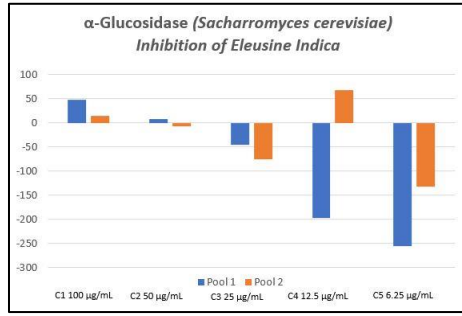

Figure 10. Percentage inhibition of Eleusine indica pooled fractions against α-glucosidase

DISCUSSION

Diabetes is distinguished by elevated levels of sugar in the blood that may lead to fatal complications like failure of the system and/or deterioration of the eyes, kidneys and various diseases affecting the cardiovascular system. a-glucosidase as a key enzyme for the digestion of carbohydrates has been known to be the target for postprandial hyperglycemia therapy which is found to be the earliest problem in metabolism activity that occur. Dietary carbohydrates like starch are one of the major sources of blood sugar. It is hydrolyzed by pancreatic a-amylase and further by a-glucosidase by why which it is absorbed in the small intestine. Therefore, the main focus of the treatment is to reduce the fluctuations in blood sugar levels and other complications related to it. Certain drugs that work by retarding the uptake of dietary carbohydrates and suppress postprandial hyperglycemia are useful for treating diabetic patients (Thilagam et al., 2013).

In the continuing search of the useful origin of medicinal, plants have shown to possess active compounds that are now being investigated for its use in the pharmaceutical industry (Saleem et al., 2016). In this study, the leaves of *Eleusine indica* were used to determine its a-amylase and a-glucosidase inhibitory activities and its total flavonoid content. The methanolic extract was subjected to column chromatography and obtained 35 fractions. The resulted 35 fractions were further pooled based on TLC profiling with the use of antimony chloride to obtain 5 pooled fractions. The pooled fractions with the highest total phenolic content were tested for a-amylase and a-glucosidase inhibitory activities and total flavonoid content.

Complex group of chemicals such as polyphenols that form an integral part of the human diet are distributed widely

throughout the plant kingdom. Therefore, a-amylase and a-glucosidase inhibition found in our experiments could be possible due to the polyphenol compounds present in our plant (Thilagam et al., 2013). Out of all the polyphenolic compounds, the most common group is flavonoids. Flavonoids are present in common food products, beverages, and spices that are of use since early times that caters great variety of diseases in human (Etxeberria et al., 2012). Flavonoids present in *E. indica* may potentially provide a natural source of a-glucosidase inhibitors (Thilagam et al., 2013).

CONCLUSION

In this study, it is concluded that the flavonoid content of Eleusine indica leaf extract exhibit minimal inhibitory activity against alpha-amylase and alpha-glucosidase enzymes. In comparison to the standard drug used in this study, it is observed that Acarbose possesses greater inhibitory activity compared to the activity of E. indica leaf extract at the same concentration.

RECOMMENDATION

It is recommended that the future researchers test the alpha-amylase and alpha-glucosidase inhibitory activities of E. indica in vivo procedures. Also, phytochemical screening of E. indica for the presence of other compounds that may be responsible for its a-amylase and a-glucosidase inhibition is recommended.

REFERENCES

Ali, H., Houghton, P. J., & Soumyanath, A. (2006). -Amylase inhibitory activity of some Malaysian plants used to treat diabetes ; with particular reference to Phyllanthus amarus, *107*, 449–455. https://doi.org/10.1016/j.jep.2006.04.004

Arora, S., & Itankar, P. (2018). Journal of Traditional and Complementary Medicine Extraction , isolation and identi fi cation of fl avonoid from Chenopodium album aerial parts. *Journal of Traditional Chinese Medical Sciences*, 1–7. https://doi.org/10.1016/j.jtcme.2017.10.002

Asghari, B., Salehi, P., Farimani, M. M., & Ebrahimi, S. N. (2015). ??-Glucosidase Inhibitors from Fruits of Rosa canina L. *Records of Natural Products*, *9*(3), 276–283. https://doi.org/10.1590/S1415-47572000000100010

Bajpai, V. K., Majumder, R., & Park, J. G. (2016). Isolation and

purification of plant secondary metabolites using column-chromatographic technique, 844–848. https://doi.org/10.3329/bjp.v11i4.28185

Benalla, W., Bellahcen, S., & Bnouham, M. (2010). Antidiabetic Medicinal Plants as a Source of Alpha Glucosidase Inhibitors, (212), 247–254.

Chakrabarti, R., Sigh, B., VN, P., Vanchhawng, L., & Thirumurugan. (2014). Screening of Nine Herbal Plant for Inhibition α-Amilase Inhibition. *Asian Journal of Pharmaceutical and Clinical Research*, *7*(4), 84–89.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-Based Complementary and Alternative Medicine*, *2014*. https://doi.org/10.1155/2014/253875

Condori-peñaloza, A. E. M., & Costa, S. S. (2016). Characterization of Main Phenolic Compounds in Eleusine indica L . (Poaceae) by HPLC-DAD and 1H NMR, *10*(6), 2016.

de Sales, P. M., de Souza, P. M., Simeoni, L. A., Magalhães, P. de O., & Silveira, D. (2012). α-amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy and Pharmaceutical Sciences*, *15*(1), 141–183. https://doi.org/10.18433/J35S3K

Etxeberria, U., de la Garza, A. L., Campión, J., Martínez, J. A., & Milagro, F. I. (2012). Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert Opinion on Therapeutic Targets*, *16*(3), 269–297. https://doi.org/10.1517/14728222.2012.664134

Fawzy, G. A., Abdallah, H. M., Marzouk, M. S. A., Soliman, F. M., & Sleem, A. A. (2008). Antidiabetic and antioxidant activities of major flavonoids of Cynanchum acutum L. (Asclepiadaceae) growing in Egypt. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *63*(9–10), 658–662. https://doi.org/10.1515/znc-2008-9-1008

Gaikwad, S. B., Krishna Mohan, G., & Sandhya Rani, M. (2014). Send Orders for Reprints to reprints@benthamscience.net Phytochemicals for Diabetes Management. *Pharmaceutical Crops*, *5*, 11–28.

He, M., Min, Jpeng, W. L., He, X. H., Li, J. X., & Peng, B. W. (2016). A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia*, *115*, 74–85. https://doi.org/10.1016/j.parint.2016.12.005

Jawad, M., Schoop, R., Suter, A., Klein, P., & Eccles, R. (2013).

Perfil de eficacia y seguridad de Echinacea purpurea en la prevención de episodios de resfriado común: Estudio clínico aleatorizado, doble ciego y controlado con placebo. *Revista de Fitoterapia*, *13*(2), 125–135. https://doi.org/10.1002/jsfa

Kalita, D., Holm, D. G., LaBarbera, D. V., Petrash, J. M., & Jayanty, S. S. (2018). Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds. *PLoS ONE*, *13*(1), 1–21. https://doi.org/10.1371/journal.pone.01910

Kumar, A. S., Kavimani, S., Narasimha, K., Pradesh, A., Nehru, J., & Pradesh, A. (2012). Anti-diabetic and anti-hyperlipidemic effects of methanol extracts of Chloris barbata (SW .) in Streptozotocin- induced diabetic rats Department of Chemistry , Jawaharlal Nehru Engineering College , Jawaharlal Nehru, *2*(4), 1346–1353.

Mohd Abdul Alim, N. A. S., Sulaiman, A. Z., & Ajit, A. (2016). Application of ultrasound on the extraction of Vitexin from Ficus deltoidea leaves. *ARPN Journal of Engineering and Applied Sciences*, *11*(4), 2199–2204. https://doi.org/10.1016/j.jep.2012.05.062

Ojewunmi, O., Oshodi, T., Ogundele, O., Micah, C., & Adenekan, S. (2013). Evaluation of the Anti-Diabetic and Antioxidant Activities of Aqueous Extracts of Morinda lucida and Saccharum officinarum Leaves in Alloxan-Induced Diabetic Rats. *International Journal of Biochemistry Research & Review*, *3*(33), 266–277. https://doi.org/10.9734/ijbcrr/2013/5043

Okokon, J. E., Odomena, C. S., Effiong, I., Obot, J., & Udobang, J. A. (2010). Antiplasmodial and antidiabetic activities of Eleusine indica. *International Journal of Drug Development and Research*, *2*(3), 493–500. https://doi.org/doi:10.1086/597786

Okwo, F. (2014). Fractionation and determination of total antioxidant capacity , total phenolic and total flavonoids contents of aqueous , ethanol and n-hexane extracts of Vitex doniana leaves, *13*(5), 693–698. https://doi.org/10.5897/AJB2013.13225

Ong, S., Nalamolu, K., & Lai, H. (2017). Potential lipid-lowering effects of *Eleusine indica* (L) Gaertn. Extract on high-fat-diet-induced hyperlipidemic rats. *Pharmacognosy Magazine*, *13*(49), 1. https://doi.org/10.4103/0973-1296.203986

Opoku Amoah, D. (2017). Antiurolithiatic Potential of <i>Eleusine indica</i> Linn. (GOOSE GRASS) Root Extract on Ethylene Glycol Induced Nephrolithiasis in Rattus norvegicus (ALBINO RATS). *Biomedical Sciences*, *3*(5), 99. https://doi.org/10.11648/j.bs.20170305.13

Pattanayak, S., & Maity, D. (2017). Short Communication USE OF ELEUSINE INDICA (L .) GAERTN . (KECHILA GHAS) AS AN ANTIPYRETIC MEDICINE OF HERBIVORES, *7*, 94–96.

Peng, X., Zheng, Z., Cheng, K. W., Shan, F., Ren, G. X., Chen, F., & Wang, M. (2008). Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. *Food Chemistry*, *106*(2), 475–481. https://doi.org/10.1016/j.foodchem.2007.06.016

Sabiu, S., O'Neill, F. H., & Ashafa, A. O. T. (2016). Kinetics of α-amylase and α-glucosidase inhibitory potential of Zea mays Linnaeus (Poaceae), Stigma maydis aqueous extract: An in vitro assessment. *Journal of Ethnopharmacology*, *183*, 1–8. https://doi.org/10.1016/j.jep.2016.02.024

Sambandam, B., Thiyagarajan, D., Ayyaswamy, A., & Raman, P. (2016). Extraction and isolation of flavonoid quercetin from the leaves of Trigonella foenum-graecum and their anti-oxidant activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(6), 120–124. https://doi.org/10.1136/tobaccocontrol-2013-051502

Shim, Y. J., Doo, H. K., Ahn, S. Y., Kim, Y. S., Seong, J. K., Park, I. S., & Min, B. H. (2003). Inhibitory effect of aqueous extract from the gall of Rhus chinensis on alpha-glucosidase activity and postprandial blood glucose. *Journal of Ethnopharmacology*, *85*(2–3), 283–287. https://doi.org/10.1016/S0378-8741(02)00370-7

Tahrani, A. A., Bailey, C. J., Del Prato, S., & Barnett, A. H. (2011). Management of type 2 diabetes: New and future developments in treatment. *The Lancet*, *378*(9786), 182–197. https://doi.org/10.1016/S0140-6736(11)60207-9

Tan, G. H. (2015). Diabetes Care in the Philippines. *Annals of Global Health*, *81*(6), 863–869. https://doi.org/10.1016/j.aogh.2015.10.004

Temidayo, A. R. (2013). Extraction and Isolation of Flavonoids Present in the Methanolic Extract of Leaves of Acanthospermum HispidiumDc. *Global Journal of Medicinal Plant Research*, *1*(1), 111–123. Retrieved from http://www.aensiweb.com/old/GJMPR/2013/111-123.pdf

Thilagam, E., Parimaladevi, B., Kumarappan, C., & Chandra Mandal, S. (2013). α-Glucosidase and α-Amylase Inhibitory Activity of Senna surattensis. *JAMS Journal of Acupuncture and Meridian Studies*, *6*(1), 24–30. https://doi.org/10.1016/j.jams.2012.10.005

Tutor, J. T., & Chichioco-hernandez, C. L. (2018). Angiotensin-Converting Enzyme Inhibition of Fractions from Eleusine indica Leaf Extracts, *10*(1), 25–28.

Tyagi, B., Trivedi, N., & Dubey, A. (2017). αα α -Amylase

Inhibitor : A Compelling Plant Defense Mechanism Against Insect / Pests α -Amylase Inhibitor : A Compelling Plant Defense Mechanism Against Insect / Pests, (March 2014).

Value, S. J. R. I., Siddiqui, A. A., Siddiqui, S. A., Ahmad, S., Siddiqui, S., Ahsan, I., & Sahu, K. (2013). Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands Diabetes : Mechanism , Pathophysiology and Management-A Review, *5*(2), 1–23.

Wu, H., & Xu, B. (2014). Inhibitory effects of onion against α-glucosidase activity and its correlation with phenolic antioxidants. *International Journal of Food Properties*, *17*(3), 599–609. https://doi.org/10.1080/10942912.2012.654562

Yang, J. P., He, H., & Lu, Y. H. (2014). Four flavonoid compounds from phyllostachys edulis leaf extract retard the digestion of starch and its working mechanisms. *Journal of Agricultural and Food Chemistry*, *62*(31), 7760–7770. https://doi.org/10.1021/jf501931m

Yang, W., Dall, T. M., Beronjia, K., Lin, J., Semilla, A. P., Chakrabarti, R., … Petersen, M. P. (2018). Economic costs of diabetes in the U.S. in 2017. *Diabetes Care*, *41*(5), 917–928. https://doi.org/10.2337/dci18-0007