In Vitro Mammalian Alpha-glucosidase Inhibitor Screening of Selected Plant Materials from Siba-o, Calabanga, Camarines Sur

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RESEARCH ARTICLE

Abstract

Background and Objectives: Diabetes mellitus type 2 (DM2) remains a health threat to Filipinos. According to the International Diabetes Federation 2014, the Philippines is one of the emerging DM2 hotspots with an estimated prevalence rate of around 3.2 million cases (5.9%) between 20 to 79 years old. In line with the acceptance of Filipino patients with the utilization of herbal medicine as an effective alternative for treatment of their ailments, some of the selected plant materials from Siba-o, Calabanga, Camarines Sur were explored for mammalian intestinal alpha-glucosidase inhibition *in vitro* to develop new herbal drug candidates that are effective, safe, and more affordable.

Methodology: Exhaustive maceration using absolute ethanol was performed to extract the phytoconstituents from the plant matrix. *In vitro* alpha-glucosidase inhibition assay using spectroscopic method (96 well plates) was carried out to analyze the mammalian α-glucosidase inhibition of the different plant samples, IC_{50} was determined from the generated linear regression extrapolated from concentrations-% inhibitions plot. Thin Layer Chromatography (TLC) bioautography was employed to identify the presence of flavonoids, tannins, essential oil, reducing sugar, coumarin, anthraquinones, anthrones, steroids, alkaloids, and peptides.

Results: From the 98 crude plant samples extracted, the ethanolic extracts of *Melothria sp.* stem with leaves showed a concentration-dependent inhibition activity towards mammalian α-glucosidase from rat intestine acetone powder with IC_{50} values of 49.24 ppm. Tannins, flavonoids, essential oils, and indoles were detected from TLC bioautography that may be responsible for the bioactivity.

Conclusions and Recommendations: The results demonstrated the potential utilization of some plant samples as an alternative herbal drug. However, only *Melothria sp.* crude leaves and stem extract (SB32LS) showed a concentration-dependent activity and further studies must be done to isolate the metabolites responsible for the activity through activity-guided isolation.

Keywords: *Type 2 diabetes mellitus, mammalian α-glucosidase inhibition, Melothria sp., Siba-o, Calabanga, Camarines Sur*

Introduction

Diabetes mellitus is a group of metabolic syndromes characterized by absolute or relative deficiencies in insulin release and is associated with chronic hyperglycemia which can lead to macrovascular, microvascular, and neuropathic disorder [1,2]. The prevalence, medication cost, and disabling complications, such as stroke, amputation, blindness, and end-stage kidney caused by type 2 diabetes are threats to human health [3,4]. According to the International Diabetes Federation 2014, the estimated prevalence of DM2 in the Philippines was 3.2 million cases with 5.9% prevalence rate between 20 to 79 years old [5]. Therefore, it is essential for early DM treatment and reduction of chronic complications development to control post-prandial plasma glucose level. Inhibitors of α-glucosidase, such as acarbose, miglitol, and voglibose are oral therapeutic agents that delay the complex carbohydrates digestion by inhibiting polysaccharide digesting enzymes including pancreatic α- amylase and α-glucosidase. These commercial drugs effectively display antihyperglycemic activity. However, the utilization of the mentioned agents are limited due to gastrointestinal side effects, such as vomiting,

flatulence, abdominal cramps, and diarrhea [6,7]. Hence, researchers seek for alternative drugs that exert an α-glucosidase activity without the gastrointestinal discomfort in line with the acceptance of Filipino patients of herbal medicine usage.

The exploration of natural products has a profound contribution on the improvement of human health [8]. Botanically-derived compounds, as routes to drug discovery, continue to be a proven way to drug development, especially in developing countries where people from the marginalized sector usually depend on herbal medicine to treat their ailments [9]. As a biodiversity system, Mt. Isarog, a distinct sub-region of the Southern Luzon, has an unexploited opportunity of finding novel therapeutic agents by bioactivity-guided screenings [10]. Approximately 1,300 flora have been reported and more studies are ongoing. Several species of endemic reptiles, birds, and mammals have been noted in Mt. Isarog since 1961 and have been the cause of biodiversity conservation. The International Union for the Conservation of Nature (IUCN) and World Wildlife Fund (WWF) declared Mt. Isarog as one of the Centers of Plant Diversity and Endemism in the Philippines and Centers for Animal Diversity and Endemism, being part of the Luzon faunal region. The municipalities surrounding Mt. Isarog, such as Naga, Calabanga, Tinambac, Goa, Tigaon, Ocampo, and Pili are parts of a rich pool of the indigenous and endemic species [11].

The aim of this study was to conduct a preliminary investigation on α-glucosidase inhibitory activity of selected botanical materials. The research only covered bioactivity screening and identification of bioactive metabolites using TLC bioautography. Alpha-glucosidase from rat intestine acetone powder was chosen due to the relative correlation of enzymatic source to mammalian α-glucosidase in vivo [12].

Methodology

Collection, Identification and Processing of Selected Plant Materials

The plant materials collected from a private lot located at Siba-o, Calabanga, Camarines Sur were washed in running tap water, their extraneous matters were removed by garbling, and then hanged on a fishnet basket to dry. The samples were identified at Ateneo de Naga University, University of the Philippines-Diliman, and University of Santo Tomas (see Table 3 for sample identity). The voucher specimens of all plant samples in the study were deposited in the respective institutions.

The dried samples (crisp to touch) were comminuted using a household osterizer. The powdered materials were macerated thrice for three successive days with sufficient amount of absolute ethanol with constant shaking using an improvised mechanical shaker. The resulting filtrates were all combined and concentrated using a rotary evaporator with a temperature maintained at about 40°C to recover the solid residue. Crude extracts were stored at -8.0°C until further analysis.

Phytochemical Screening

The phytochemical screening of crude extract was based on methods reported by Geuvara, B. 2007 [13]. Thin layer chromatography bioautography was performed by developing the chromatogram of crude extract (20 µL) using the optimized solvent system in Silica Gel GF_{254} TLC plates with aluminum backing. The developed TLC chromatogram was air dried and sprayed with the solutions from Table 1 for analysis of metabolites:

Table 1. *Analysis of Metabolites*

Spray Reagents	Metabolite(s) tested
Potassium ferricyanide-ferric chloride followed with 2M HCl	Phenolic compound, tannins, and flavonoids
3, 5- Dinitrobenzoic acid: Kedde reagent	Cardenolides
Methanolic Borntrager reagent	Coumarins, anthraquinones, anthrones, and phenolic compound
Magnesium acetate solution	Antharquinones
Vanillin-sulfuric acid	Essential oils, higher alcohols, phenols, and steroids
Antimony (III) chloride	Flavonoid
Dragendorff's reagent: muniermachebouef	Alkaloid
α-napthol-sulfuric acid	Sugar
Van Urk-Salkowski	Indoles
Ninhydrin	Peptides

Alpha-glucosidase Inhibitor Screening

Assay of Mammalian Alpha-glucosidase Inhibition

Spectrophotometric endpoint analysis of mammalian α-glucosidase inhibition was performed according to the reported method of Mohamed S.S., Hansi, D., and Kavitha T.P., 2011 [14] and modified mammalian enzyme preparation by Kang, B.H., Racicot K., Pilkenton and Apostolidis, E., 2015 [15]. The crude plant extracts were dissolved in 100% dimethylsulfoxide at different concentrations. One millimolar (1 mM) p-nitrophenyl glucopyranoside (substrate) was prepared in 50 mM pH 6.8 phosphate buffer with 100 mM NaCl. In 96-well flat-bottom plates, 50 µL of phosphate buffer saline solution and 25 µL of varying concentrations (10, 20 and 100 ppm) of crude plant extracts were added followed by 50 µL of 1 mM substrate. After addition of the solutions to individual wells, the microplate was incubated for 5 min at 37°C inside the microplate reader. The sample plate was then removed after incubation and 25 µL of 33.33 mg/mL α-glucosidase from rat intestine acetone extract was added. The enzymes were prepared by extracting fifty milligrams (50 mg) of rat intestine acetone powder with 1.5 mL of 50 mM pH 6.8 phosphate buffer with 100 mM NaCl. The mixture was vortex-mixed, sonicated for 5 minutes and centrifuged for 30 minutes. at 10,000 X g, 4°C. The resulting supernatant was used for the assay. The reaction mixture was reincubated at 37°C for 30 minutes. and the reaction was terminated with addition of 0.1 M sodium carbonate solution. Phosphate buffer saline with dimethylsulfoxide was used as negative control. Acarbose secondary standard to European Pharmacopeia was used as positive control. To subtract the absorbance due to colored plant extracts, the wells containing buffer and test samples were allocated with separate wells. In addition, the absorbance due to the yellow color of crude mammalian enzyme was also eliminated by adding separated wells containing buffer and enzymes. The spectral changes were monitored by analyzing the para-nitrophenol released from the substrate at 405 nm using BMG® microplate reader and the data were generated through Omega program version 3.10 R6. The assay was performed in triplicate.

Data Analysis

The percentage inhibition was calculated using the equation below:

$$\% \, Inhibition = \frac{[(\text{Ac-Ab})-(\text{As-Ab})]}{\text{Ac-Ab}}$$

Where Ac= negative control, As= sample absorbance, and Ab= background absorbance (to eliminate absorbance made by plant pigments from crude plant extract and yellowish appearance from mammalian enzyme).

The statistical difference between the alpha-glucosidase percentage inhibitions of the different test samples was analyzed using Kruskal-Wallis test followed by Mann-Whitney U test. Concentrations against % inhibition graphs were constructed for plant samples and the IC_{50} of the plant extracts were extrapolated using linear regression.

Results and Discussion

Metabolites Identified via TLC-Bioautography

Out of 98 crude extracts from 41 plant species (Table 3), SB32LS was the only plant material that exhibited a concentration-dependent activity. Due to the remarkable outcome of enzyme inhibition assay of SB32LS, this was the only sample tested for the presence of phytochemicals using TLC bioautography (Table 1). Phenolic compounds were detected on SB32LS. Flavonoid and tannins are ubiquitous in nature and are found to have benefits on human health such as antioxidant (free radical scavenging), antimicrobial, inhibition of triglycerides deposition, and anti-inflammatory. Reduction of non-communicable diseases, such as diabetes, cardiovascular diseases, and cancer had been reported [16]. Many flavanoids weakly inhibit rat small intestine α-glucosidase due to the presence of isoflavone (genistein and daidzein with mixed type of inhibition) and anthocyanidin group (cyanidin) in vitro [17]. It was reported that flavonoid glycosylation diminishes the α-glucosidase inhibitory activity depending on the class of sugar moiety and conjugation site. Increase in polarity, molecular size and transfer to the non-polar structure are the proposed reasons for decreasing inhibition activity after glycosylation [18]. On the other hand, tannins exert anti-diabetic activity by inducing insulin-sensitive tissues, retarding blood glucose absorption, and acting as non-specific inhibitors of α-amylase and α-glucosidase. The proanthocyanidin content of tannins determines the extent of inhibitory effect against the enzyme [19]. Although tannins have inhibitor effect against carbohydrate-digesting enzymes, this phytoconstituent has low impact and priority for drug discovery [20]. Bands with Rf values of 0.37, 0.79, and 0.84 were interpreted as phenolic compounds due to positive reaction with ferric chloride-potassium ferricyanide solution. No available literatures could suggest a probable mechanism of essential oil and indole-containing compound in glucosidase

inhibition. Regardless, researches of essential oil from another medicinal plant *Ocimum sanctum* was reported as a good antidiabetic agent due to its capability to lower blood sugar, triglycerides, cholesterol, LDH, alkaline phosphatase, GPT, and GOT in blood serum [21]. The presence of essential oils using vanillin-sulfuric acid solution was detected with Rf values of 0.26, 0.37, 0.84, and 0.90. Indole is a parent compound of multiple compounds that occur in nature, such as tryptophan, serotonin, and strychnos alkaloid. Indole as core nucleus of new therapeutic molecule may replace many approved drugs in the future, especially in disorders, such as cancer (cediramib, apazaquinone), hypertension (perindopril, pindolol), HIV (ateviridine, delavirdine) and depression (binedaline, amedalin) [22]. The observed band with Rf value of 0.79 was positive for the presence of indole related compound.

Enzyme Assay Screening

The results of the screening of the selected plant materials using in vitro mammalian alpha-glucosidase inhibitor are summarized in Table 3. Among the 98 crude extracts analyzed, SB13, SB17S, SB17L, SB32LS, SB48, SB49 Fr, SB49L, SB50L, and SB54L were able to inhibit rat intestine alpha-glucosidase in vitro. However, some of these test samples (SB13, SB17S, SB17L, SB49Fr, and SB49L) were not analyzed for higher concentrations (from 20 ppm to 100 ppm) due to limited availability of biomass for the screening. *Grammatophyllum scriptum* (SB13W) (Family: Orchidaceae) and *Pueraria phaseoloides* (SB17S and SB17L) (Family: Fabaceae) were also active glucosidase inhibitors at 20ppm with 49.70%, 48.28%, and 52.29% inhibition respectively. These plant species were only utilized for ornamental

Table 2. *Phytochemical test results of SB32SL using TLC bioautography.*

Rf value	Metabolite detected from SB32LW
0.26	(+) Essential oil
0.37	(+) Essential oil (+) Tannins (+) Glycosidic flavonoids
0.79	(+) Indoles (+) Tannins (+) Glycosidic flavonoids
0.84	(+) Essential oil (+) Glycosidic flavonoid (+) Indoles
0.90	(+) Essential oil

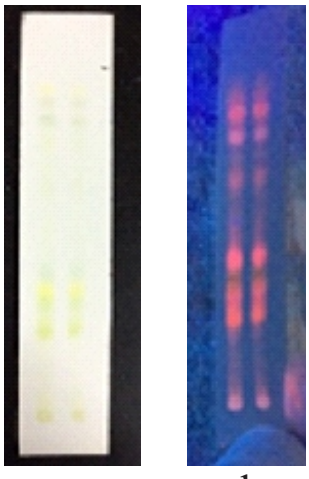

Figure. *(a) Thin layer chromatogram of SB32LS developed on normal phase TLC plate using 8:2 $CHCl_3$: EtOAc; (b) Thin layer chromatogram of SB32LS under 366 nm*

purpose [23] and as ruminant feeds on livestock animal [24] respectively. Further *in vitro* and *in vivo* analyses of SB13, SB17S, and SB17L are needed to verify the potential use of these plant species for diabetes management. *Antidesma bunius* fruits were previously reported to have a relatively low inhibition in yeast glucosidase (22.0% at 1mg/mL) but after partial purification using column chromatography, the samples showed a slight improvement in bioactivity (Fraction 4 from column chromatography purification yielded a 45.0% yeast glucosidase inhibition). Flavonoids, tannins, and sugars are phytoconstituents that were detected on crude and semi-purified samples [25]. The contradicting results of this study may be due to the difference of enzyme source (Type I for baker's yeast and Type II mammals based on primary structure difference) used in the assay [12]. In addition, internal and external factors affect metabolite production, such as, physiological variations (age of plant during collection), environmental conditions (climate, soil condition, water, and mineral), geographical variations, genetic factors, and handling of plant materials (type of plant material, timing of collection, and storage condition) can cause the disparity of *in vitro* assay results and other biological tests that will be conducted [26,27].

At the concentration of 10 ppm and 20 ppm, there was no statistical difference between acarbose (standard drug), SB32LS, Sb48, SB50L, and SB54L ($H_{statistical}<H_{critical}$). However, there was a statistical difference among test groups at 100 ppm concentration ($H_{statistical}>H_{critical}$). *Cleome rutidosperma* DC (Family: Capparidaceae) is a low growing herb (up to 70 cm) that usually grows in grassy places and wastelands. The roots, leaves, and seeds of Cleome species are utilized as carminative, vesicant, anthelminthic, rubifacient, stimulant, and antiscorbutic [28]. All concentrations of SB48 and acarbose tested have no significant difference in inhibiting alpha-glucosidase ($U_{statistical}<U_{critical}$). Carbohydrates, steroids, alkaloids glycosides, flavonoids, tannins, saponins, lipids, and proteins are metabolites previously extracted by other researchers that may contribute to the bioactivity of the test sample [29].

Mann-Whitney U test revealed that SB48, SB54L, and Acarbose had no significant differences at 10 ppm and 20 ppm ($U_{statistical}< U_{critical}$). However, at 100 ppm, there was a significant difference between the enzyme inhibitions between the test groups ($U_{statistical}>U_{critical}$). *Commelina diffusa* (Family: Commelinaceae) is a herbal medicine used for treatment of different ailments, such as hemorrhoids, ophthalmia, laryngitis, sore throat, rheumatoid arthritis, gonorrhea, common cold, leprosy, tuberculosis, dysentery and cough [30,31]. Various metabolites, such as reducing sugar, tannins, alkaloids, phytosterols, flavonoids, and triterpenoids are reported in the sample that markedly affect the activity of the sample [30]. On the other hand, *Gliricidia sepium* (Family: Fabaceae) is composed of essential oil [32], triterpenoid [33], coumarin [34] and phenolic compounds [35]. Madre de Cacao was used as timber, cover crop, soil improver, and ruminant fodder but non-ruminant fed on this plant display sign of toxicity. Individual parts of *G. sepium* were reported to be toxic to humans when ingested with maize or cooked rice although the mechanism of toxicity was not yet confirmed [36].

3 4 5 6

Figure. *(3) Melothria sp. leaves; (4) Melothria sp. flower; (5) Melothria sp. unripe fruit, and (6) Melothria sp. ripe fruit*

Table 3. *Results of the screening of the selected plant materials using in vitro mammalian alpha-glucosidase inhibitor*

Sample Identification		% inhibition against mammalian alpha-glucosidase (mean +SD, n=3)		
Sample Code	Botanical source/Drug	10 ppm	20 ppm	100 ppm
STD	Acarbose secondary standard (EurPhar)	39.88+4.54	45.73+1.17	58.58+1.37
SB1S	*Stachytarpheta jamaicensis*	35.44+2.79	32.72+1.90	-
SB1L	*Stachytarpheta jamaicensis*	35.83+2.89	33.21+2.34	-
SB2L	*Eletophantopus scaber*	32.28+2.02	34.14+1.47	-
SB3L	*Mimosa pudica*	36.72+3.04	41.40+12.41	-
SB4L	*Cyathula prostata*	36.71+5.37	35.43+2.69	-
SB7L	*Zingiber zerumbet*	32.86+3.58	39.60+0.76	-
SB7R	*Zingiber zerumbet*	32.82+3.82	38.36+1.54	-
SB8R	*Solanum torvum*	36.82 +5.80	40.82+7.00	-
SB8L	*Solanum torvum*	31.51+12.84	36.78+0.65	-
SB9L	*Hyptis suaveolens*	32.56+4.89	37.21+6.92	-
SB9Fr	*Hyptis suaveolens*	27.84+6.12	35.65+1.91	-
SB10Fl	*Senna torra*	30.66+3.41	43.09+5.82	-
SB10L	*Senna torra*	35.08+5.99	39.23+0.95	-
SB11L	*Ageratum conyzoides*	29.07+2.47	46.45+0.96	-
SB13L	*Grammatophyllum scriptum*	32.01+1.21	49.70+4.94*	-
SB16L	*Croton tiglum*	34.25+2.01	45.15+5.01	-
SB17S	*Pueraria paseloides*	33.57+1.50	48.28+4.92*	-
SB17L	*Pureraia phaseloides*	31.67+1.87	52.29+4.56*	-
SB18L	*Urena sinuata*	34.19+0.77	50.83+6.14	47.51+7.61
Sb18S	*Urena sinuata*	32.58+1.48	39.80+3.64	-
SB19L	*Stachytarpheta indica*	34.24+1.15	38.57+1.11	-
SB19S	*Stachytarpheta indica*	34.72+3.22	23.64+6.32	-
SB20L	*Sida sp.*	34.87+1.72	41.82+2.17	-
SB24S	*Asystasia gangetica*	33.72+1.02	35.72+1.02	-
SB25F	*Canna edulis*	32.14+3.96	32.40+6.16	-
SB26W	*Macrolepia speluncae*	36.75+0.86	34.69+5.07	-
SB27S	*Hoya sp.*	31.25+1.85	31.46+1.91	-
SB29R	*Maranta arundicacea*	33.82+3.13	31.66+4.42	-
SB29S	*Maranta arundicacea*	40.18+1.82	40.32+5.23	-
SB32LS	*Melonthia sp. (Melothria palida- unofficial)*	32.55+3.80	45.69+4.99	72.24+1.74
SB33S	*Phyllanthus debilis*	26.90+1.02	34.41+7.00	-
SB42S	*Eleotheranthus ruderalis*	35.52+2.80	33.46+2.91	-

SB44L	*Crytococcum ruderalis*	28.59+3.22	37.00+1.88	-
Sb46L	*Chomolaena odorata*	33.08+0.85	37.47+0.98	-
Sb47L	*Amorphophallus paeonifolius*	34.97+1.18	35.08+2.73	-
Sb48	*Cleome rutidosperma*	32.91+4.06	55.59+8.53	58.47+11.41
SB49L	*Antidesma bunius*	33.87+3.81	54.43+1.77*	-
SB49Fr	*Antidesma bunius*	35.19+3.81	54.77+6.10*	-
SB50L	*Commelina diffusa*	33.82+5.86	48.91+4.07	50.52+3.77
Sb52L	*Ipomea alba*	14.91+3.25	33.74+5.64	-
Sb52S	*Ipomea alba*	33.14+6.15	44.94+4.42	-
SB53L	*Pennisetum purpureum*	43.14+0.95	32.89+4.16	-
SB53S	*Pennisetum purpureum*	35.77+0.97	36.00+4.14	-
SB54L	*Gliciridia sepuim*	48.49+5.2	54.76+8.16	53.37+2.11
SB55L	*Pueraria phaseoloides*	29.51+3.49	31.03+1.42	-
SB57F	*Amaranthus vividus*	33.60+3.08	42.42+1.07	-
SB57L	*Amaranthus vividus*	34.53+2.35	36.68+5.17	-
SB57S	*Amaranthus vividus*	15.00+2.18	38.56+3.03	-
SB58L	*Centella asiatica*	35.88+1.98	32.24+5.91	-
SB59Fr	*Justica betonica*	36.50+3.10	41.00+8.29	-
SB59L	*Justica betonica*	34.41+3.27	36.61+8.20	-
SB59S	*Justica betonica*	36.22+3.8	43.37+2.40	-
SB60Fl	*Urtica sp.*	35.07+2.95	37.00+2.57	-
SB60S	*Urtica sp.*	31.14+2.42	36.91+13.85	-
SB60L	*Urtica sp.*	29.16+4.14	38.64+4.42	-
SB61F	*Crossandra infondibuloformis*	36.56+0.70	27.21+2.67	-
SB64R	*Typlonium sp.*	37.10+2.33	40.37+0.97	-
Sb64S	*Typlonium sp.*	36.40+1.83	43.51+3.03	-
SB64L	*Typlonium sp.*	34.78+3.58	35.61+2.43	-
SB65L	*Caladium bicolor*	35.57+0.46	39.59+4.48	-
SB65R	*Caladium bicolor*	34.43+5.32	40.66+2.08	-
SB70L	*Canarium ovatum*	34.18+2.59	35.72+0.75	-
SB70S	*Canarium ovatum*	29.62+5.41	32.46+4.58	-
SB73Fl	*Sterlitizia reginae*	34.42+4.42	34.90+1.65	-
SB73L	*Sterlitizia reginae*	33.87+2.17	36.57+3.94	-
SB73S	*Sterlitizia reginae*	29.77+1.71	32.64+3.47	-

*not tested at 100 ppm due to limited amount of biomass available for the screening study or seasonal availability of the plant materials for activity guided isolation
- not tested at 100 ppm because the samples failed to exert at least 45% above inhibition at 20 ppm
L(leaves), Fr(Fruit), S (Stem), R(Root), SL(Stem and Leaves) and W (Whole plant)

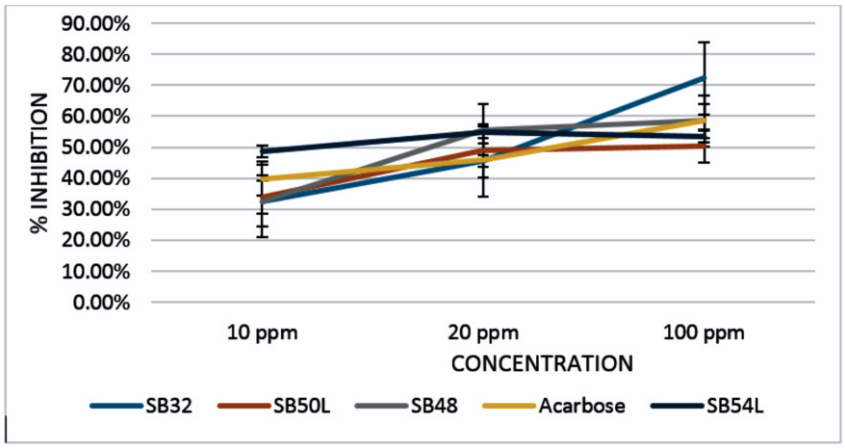

Figure 7. *Percentage inhibitions of SB32LS, SB50L, SB48, SB54L and acarbose at different concentrations against rat intestine acetone powder*

Melothria sp. leaves with stem (SB32LS) exhibited concentration-dependent inhibition activity against mammalian alpha-glucosidase in vitro. *Melothria sp.* is an undocumented plant species from Cucurbitaceae. The Genus Melothria is characterized by climbing slender herbs with tendrils simple. The leaves are deltoid-ovate, base truncate or cordate, entire or lobed, usually scabrous while the flowers are small, monoecious, pedicellate, and axillary. Male flowers have three stamens inserted in the middle of the calyx-tube while the female flowers have an oblong ovary. The fruit is globose to ellipsoid or fusiform and the numerous seeds inside are small [37]. To resolve the species of the reported plant sample, additional study using a new method for plant verification such as DNA barcoding hypenated with traditional method-voucher specimen preparation will help to shed some light regarding the identity of the plant of interest.

Melothria sp. at 10 ppm and 100 ppm had no statistically significant evidence at α=0.05 to show that the treatment from plant extracts are not the same as the reference drug, acarbose in inhibiting the intestinal rat α-glucosidase ($U_{critical} = U_{statistical}$). In contrast with the outcome of 20 ppm statistical analysis, there was no evidence to conclude that the 2 treatment groups differ in enzyme inhibition ($U_{critical}<U_{statistical}$). The concentration-dependent activity of SB32LS leads to further analysis with respect to alpha-glucosidase inhibition by IC_{50} determination. The crude *Melothria sp.* extract exerts inhibition at 49.24 ppm. The calculated IC_{50} of the *Melothria sp*. was lower than acarbose IC_{50}=53.54 ppm due to synergism or additive activity of different compounds from crude extract. Isolation of active compound from SB32LS is recommended to verify the superiority of the test samples over acarbose. Mohamed Sham Shihabudeen *et al.*, 2011 reported that acarbose had an IC_{50} value of 36.89 µg/mL in 80 µL reaction mixture against rat α-glucosidase. These results proved that enzyme concentration was an important parameter for in vitro assay as published by Shai *et al.*, 2011 [38]. In addition, the scanned absorbance spectrum of crude enzyme (Figure 8) had the same lambda max of p-nitrophenol (γmax=405), hydrolysis product of PnPG (substrate). Failure to account the enzyme absorbance from the absorbance of reaction mixture may mask the inhibition activity of the test samples leading to lower calculated inhibitory effect.

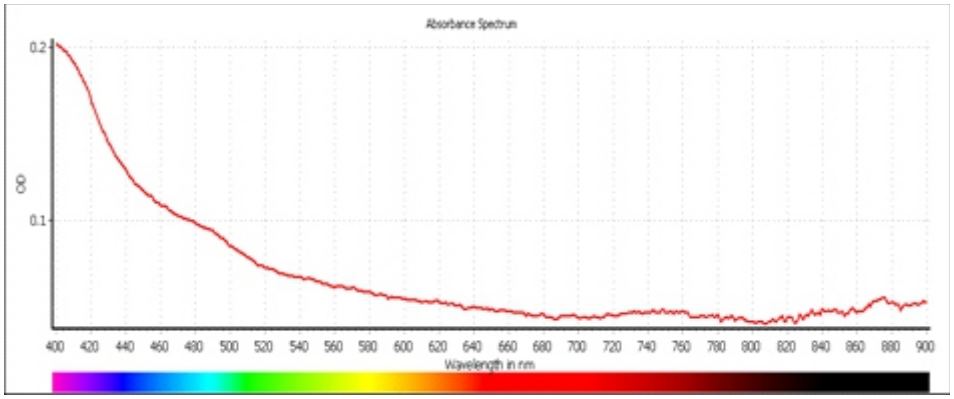

Figure 8. *Absorbance spectrum of crude α-glucosidase from rat intestine acetone powder showing that the lambda max was at the same range of p-nitrophenol (product of PnPG hydrolysis)*

Conclusion

Melothria sp. leaves with stem extract showed a concentration-dependent activity against mammalian intestinal alpha-glucosidase. Tannins, glycosidic flavonoid, essential oils, and indoles were the identified compounds that might be responsible for the observed antidiabetic activity. This is the first report of potential bioactivity of *Melothria sp.* crude extract.

Recommendation

The species of Melothria (SB32LS) must be determined using DNA barcoding together with traditional voucher specimen preparation for proper documentation of the plant species. Also, an activity guided isolation must be specifically executed to identify the active compound(s) with the α-glucosidase inhibitory effect hyphenated with structure elucidation to characterize the compound of interest.

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