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Investigation of the Bioactive Principles and α -Glucosidase Inhibitory Effect of some Myanmar Traditional Medicinal Plants

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Abstract

This research is focused on the evaluation of α -glucosidase inhibitors from eight Myanmar traditional medicinal plants such as *Ammomum xanthoides* Wall., *Commelina communis* Linn., *Crataeva religiosa* Forst., *Eupatorium odoratum* Linn., *Gynura procumbens* (Lour.) Merr., *Momordica charantia* Linn., *Spirulina* and *Syzygium grande* (WI) Walp.. All of these medicinal plants have been reported to possess the antidiabetic potential. In the present work, watery and ethanolic extracts, and some isolated compounds such as lupeol, lupeol acetate, friedelin, betulinic acid, gallic acid, stigmasterol, taraxasterol, β -sitosterol- β -D-glucoside, stearic acid, β -sitosterol, vitamin C, charatin and A. xanthoides essential oil were used to investigate their inhibitory effect on α -glucosidase enzyme activity. All of the selected medicinal plant extracts ($IC_{50} = 0.14 \sim 2.07 \mu\text{g/ml}$) as well as the isolated compounds ($IC_{50} = 0.47 \sim 1.91 \mu\text{g/ml}$) were found to show the α -glucosidase inhibitory activity. These values were found to be comparable with Voglibose ($IC_{50} = 0.32 \mu\text{g/ml}$), which was used as a reference in this study. Therefore these plants may be used as sources of natural α -glucosidase inhibitors in control or management of the postprandial hyperglycemia, Type 2 diabetes.

Keywords: Myanmar traditional medicinal plants, antidiabetic potential, natural α -glucosidase inhibitors, postprandial hyperglycaemia, Type 2 diabetes

1. Introduction

Nowadays, people were suffered from diabetes mellitus is rapidly increased. It is one of the six major diseases (diabetes, hypertension, malaria, diarrhoea, dysentery, tuberculosis) recorded in Myanmar. Most of the people have tried to use herbal drugs for the management or treatment of diabetes. There are two common types of diabetes : Type 1 diabetes, insulin-dependent diabetes and Type 2 diabetes, non-insulin dependent diabetes.

Postprandial hyperglycaemia plays an important role in the development of Type 2 diabetes mellitus due to the hydrolysing effect of α -glucosidase enzymes on the carbohydrates. α -Glucosidases (EC 3.2.1.20) are the intestinal enzymes bound in the brush border of the small intestines. They are the key enzymes of carbohydrate digestion.

To control or to manage postprandial hyperglycaemia, α -glucosidase inhibitors are used. α -Glucosidase inhibitors, a class of

diabetes drugs are known as "starch blockers" which function by slowing the absorption of certain simple sugar molecules in the gastrointestinal tract to reduce or to delay the sharp rise in blood glucose level after a meal. Acarbose, miglitol, voglibose and emiglitate have been approved to use as antidiabetes drugs. Acarbose, miglitol and voglibose act by competitively inhibiting the α -glucosidases, a group of key intestinal enzymes involved in the digestion of carbohydrates. They decrease both postprandial hyperglycaemia and hyperinsulinaemia, and thereby may improve sensitivity to insulin and release the stress on β -cells. Metformin or sulfonylureas (Diabinese, DiaBeta, Glynase, Micronase, Glucotrol, Glucotrol XL, Amaryl) have been reported to be more effective at managing blood glucose [1]. Numbers of research on the investigation of the effective natural α -Glucosidase inhibitors from herbals with antidiabetic potential have been reported [2, 3, 4].

In this work, eight species of Myanmar traditional medicinal plants such as *Ammomum xanthoides* Wall. (Chinbaung-phalar) seed, *Commelina communis* Linn. (Myint-kyut), *Crataeva religiosa* Forst., (Kadet) bark, *Eupatorium odoratum* Linn. (Bizat) leaf, *Gynura procumbens* (Lour.) Merr. (Pyar-hme) leaf, *Momordica charantia* Linn. (Kyet-hinn-khar) fruit, *Spirulina* (blue green algae) and *Syzygium grande* (WI) Walp. (Thabye-gyi) bark were chosen for the investigation of α -glucosidase inhibitory effect of the corresponding plant extracts as well as some of the isolated compounds from these plants, possessing the antidiabetic potential.

2. Experimental

2.1 Raw Materials

S. grande (WI) Walp. bark, *E. odoratum* Linn. leaf, *C. religiosa* Forst. bark, *M. charantia* Linn. fruit and *G.*

procumbens (Lour.) Merr. leaf were collected from Bago Township, Bago Region, *A. xanthoides* Wall. seeds from Thandaung Township, Bago Region, *C. communis* Linn. (the whole plant) from Yangon Region, and *Spirulina* (blue green algae) from Twinn Taung, Sagaing Region, Myanmar. These samples were identified at the Department of Botany, Yangon University. The collected samples were air-dried at room temperature after cleaning. These dried samples were then made into powder and the dried powder samples were separately stored in air-tight glass bottles.

Stigmasterol, taraxasterol and β -sitosterol- β -D-glucoside from *C. communis* Linn., lupeol and lupeol acetate from *C. religiosa* Forst. bark, β -sitosterol, vitamin C and β -sitosterol- β -D-glucoside from *G. procumbens* (Lour.) Merr. leaf, charatin from *M. charantia* Linn. fruit, stearic acid from *Spirulina* (blue green algae) and friedelin, betulinic acid and gallic acid from *S. grande* (WI) Walp. bark were isolated by using silica gel column chromatographic separation technique and structurally identified by modern spectroscopic methods. Essential oil from *A. xanthoides* Wall. seed was extracted by solvent extraction method. Some extracts such as 95% ethanol, watery, methanol, pet-ether, ethyl acetate extracts from the selected plant samples were also prepared. The above isolated compounds and extracts were used for this research work.

The chemicals used in this research were "British Drug House Chemical Ltd., Poole, England", "Kanto Chemical Co., Inc., Japan", and Hopkins and Williams Ltd., England".

2.2 Screening of inhibitory effect of the samples on α -glucosidase enzyme activity

(i) Isolation and identification of α -glucosidase enzyme from flint corn seeds

Imgerminated seeds of flint corn (100 g) were powdered by using a blender. The powder obtained was then suspended with 140 ml of 0.1 M acetate buffer (pH 5). After the suspended had been stirred with magnetic stirrer for 5 hrs at room temperature, it was filtered by using thin cloth and 817 ml of pale yellow crude extract was obtained.

About 204.3 g of solid ammonium sulphate were added to the crude extract under stirring. The resulting precipitate was removed by centrifuging at 10,000 rpm for 20 min. The supernatant was obtained as first filtrate. Subsequently, 188 g of solid ammonium sulphate were slowly added to the supernatant. The resulting precipitate was collected by the centrifuge at 12,000 rpm for 15 min and dried at room temperature. The crude enzyme precipitate was obtained. The supernatant is called second filtrate which was discarded.

The extracted α -glucosidase enzyme was identified as follows. 1 ml of starch and 1 ml of distilled water were added into the first test tube and allowed to stand for 30 minutes. Then 1 ml of iodine was added and deep blue colour was observed. In the second test tube, 1 ml of starch and 1 ml of enzyme were mixed and allowed to stand for 30 min. Then 1 ml of iodine was added. No colour was observed [5].

(ii) Screening of α - glucosidase effect of plant extracts and isolated compounds

The effect of plant extracts on α -glucosidase enzyme activity was investigated by determining the α -glucosidase inhibitory effect on the production of glucose from sucrose at 505 nm wavelength. This experiment was done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition and 50% inhibitory concentrations [6, 7, 8, 9].

Preparation of test sample solution

2 mg of each extract or each isolated compound and 10 ml of distilled water were thoroughly mixed by vortex mixer. The mixture solution was filtered and the stock solution was obtained.

Procedure

Firstly, the control solution was prepared by mixing 1 ml of sucrose, 1 ml of enzyme and 1 ml of DMSO with vortex mixer and incubated for 30 min at 37 °C followed by addition of glucose oxidase reagent (0.5 ml). After the incubation of the above mixture at 37 °C for 30 min, the reaction was stopped by immersing the test tube into a boiling water bath for 10 min and allowed to cool to room temperature.

Secondly, the background solution was prepared by mixing 1 ml of sucrose and 1 ml of 6% DMSO with vortex mixer according to the above procedure.

Finally, the test solution was prepared by mixing 1 ml of sucrose, 1 ml of sample solution and 1 ml of 6% DMSO with vortex mixer and incubated for 30 min at 37°C followed by addition of 1 ml of enzyme. After the incubation of the above mixture at 37 °C for 30 min, glucose oxidase reagent (0.5 ml) was added. After the incubation of the above mixture at 37 °C for 30 min, the reaction was stopped by immersing the test tube into a boiling water bath for 10 min and allowed to cool to room temperature.

The different concentrations (0.125, 0.25, 0.5, 1.0, 2.0 μ g/ml) of the sample solution were used. Absorbance of all solutions were measured by using a UV-7504 spectrophotometer at 505 nm. Voglibose, is an alpha-glucosidase inhibitor used for lowering post-prandial blood glucose levels in people with diabetes mellitus. was used as a reference.

Absorbance measurements were done in triplicate for each of the sample solutions. From the mean absorbance values, percent

inhibition of the sample on α -glucosidase enzyme activity and average percent inhibition on α -glucosidase enzyme activity were calculated by using following equations [10].

$$\% \text{ inhibition} = \frac{A_c - A - A_b}{A_c} \times 100$$

Where, % Inhibition = percent inhibition of test sample on α -glucosidase enzyme activity

A_c = absorbance of control solution

A_b = absorbance of background solution

A = absorbance of test sample solution

The IC_{50} , 50% inhibitory concentration of the sample on α -glucosidase enzyme activity was calculated by Linear Regressive Excel Program.

3. Results and Discussion

In this research, the following concepts were applied. α - Glucosidase enzyme can produce the glucose and fructose from sucrose by enzymatic hydrolysis.

Sucrose $\xrightarrow{\alpha\text{-glucosidase}}$ Glucose + Fructose

Therefore, the presence or absence of α -glucosidase enzyme inhibition effect of a sample can be demonstrated by the subsequent enzymatic production of glucose from the substrate sucrose.

If glucose is not produced from sucrose by α -glucosidase in the presence of the herbal extract or a compound, it can be inferred that the sample has the α -glucosidase inhibitory effect, i.e. it is an enzyme inhibitor. If the glucose is still formed from the sucrose by α -glucosidase enzyme in the presence of the herbal extract or compound, the herbal may not possess the α -glucosidase inhibitory effect.

The formation of glucose can be quantitatively determined by using UV-visible spectrophotometric technique. Glucose oxidase enzyme oxidizes the glucose into gluconic acid and hydrogen peroxide is also obtained. The hydrogen peroxide produced

induces oxidative condensation between phenol and 4-aminoantipyrine in the presence of peroxidase (POD), so a red colour is produced. The amount of glucose contained in a test sample is determined by measuring the absorbance of the red colour at 505 nm [10].

If the glucose amount increases, the absorbance of the red pigment will be increased. Hence, the lower the absorbance value, the lower the glucose content.

The absorbance of the red pigment formed from the glucose that produced from sucrose by α -glucosidase enzymatic hydrolysis, was found to be higher than that for the glucose produced from sucrose by α -glucosidase enzymatic hydrolysis in the presence of plant extracts. This observation showed that the extracts inhibited the α -glucosidase enzyme activity.

From the mean absorbance values, the percent inhibition of the corresponding crude extracts, isolated compounds and reference drug (Voglibose) in various concentrations: 0.125, 0.25, 0.5, 1.0, 2.0 $\mu\text{g/ml}$ on α -glucosidase enzyme activity were calculated and it was found that the % inhibition of the samples on α -glucosidase enzyme activity increased with increasing the concentrations.

From the % inhibition, the respective IC_{50} values for the plant extracts and isolated compounds were calculated and the results are respectively tabulated in Table 3.1 and Table 3.2.

According to the results shown in Table 3.1., it can be seen that the IC_{50} values for the ethanol (0.52 $\mu\text{g/ml}$) and watery extracts (0.54 $\mu\text{g/ml}$) from *C. communis*, and that for *A. xanthoides* Wall. essential oil (0.46 $\mu\text{g/ml}$) were observed to be slightly lower than that of reference drug Voglibose (0.32 $\mu\text{g/ml}$). Ethanol extract and watery extract from *E. odoratum* Linn. leaf gave 0.31 and 0.14 $\mu\text{g/ml}$ of IC_{50} values, respectively. Since the lower the IC_{50} values, the higher the

α - glucosidase inhibitory effect of the samples, *E. odoratum* Linn. leaf extracts have the highest potency followed by *C. communis* extracts, *A. xanthoides* essential oil, *S. grande* extracts, *Spirulina* extracts, *A. xanthoides* extracts, *G. procumbens* extracts, *M. charantia* EtOH extract, *C. religiosa* extracts, and then *M. charantia* fruit watery extract.

From the results listed in Table 3.2, it can be seen that all of the isolated compounds exhibited the α - glucosidase inhibitory effect. Among the isolated compound, the IC₅₀ of taraxasterol (0.47 μ g/ml) isolated from *C. communis* was found to be the lowest and to be similar to that of reference drug. Therefore the taraxasterol may possess the highest α - glucosidase inhibitory effect and found to be comparable with the effect of Voglibose. In addition, it was found that α - glucosidase inhibitory effects of betulinic acid (0.78 μ g/ml) and gallic acid (0.70 μ g/ml) isolated from *S. grande* bark were closed to each other. Lupeol (1.34 μ g/ml), lupeol acetate (1.28 μ g/ml), b-sitosterol (1.12 μ g/ml) and charatin (1.10 μ g/ml) were found to be comparable with other in α - glucosidase inhibitory effect. Friedelin (1.91 μ g/ml), Stigmasterol (1.76 μ g/ml) and b-sitosterol b-D-glucoside (1.79 μ g/ml), stearic acid (1.91 μ g/ml) and Vitamin C (1.8 μ g/ml) were found to have the similar α - glucosidase inhibitory effect. These observations are depicted with a bar graph diagram in Figure 3.1.

Table 3.1 α -Glucosidase Inhibitory Effects of Crude Extracts from the Selected Medicinal Plants

Sr. No.	Samples	IC ₅₀ (μ g/ml) of extracts	
		95% EtOH	Watery

1	<i>C. religiosa</i> bark	1.76±0.03	1.79±0.02
2	<i>G. procumbens</i> leaf	1.05±0.02	1.06±0.02
3	<i>M. charantia</i> fruit	1.04±0.02	2.07±0.03
4	<i>Spirulina</i>	0.83±0.01	0.81±0.01
5	<i>A. xanthoides</i> seed	0.83±0.01	0.89±0.01
6	<i>S. grande</i> (WI) bark	0.81±0.01	0.83±0.01
7	<i>C. communis</i>	0.52±0.01	0.54±0.01
8	<i>E. odoratum</i> leaf	0.31±0.01	0.14±0.01
9	<i>A. xanthoides</i> seed	0.43±0.02 (for essential oil)	
10	Voglibose (Reference)	0.32±0.005	

Table 3.2 α - Glucosidase Inhibitory Effects of the Isolated Compounds

Plant Sources	Isolated Compounds	IC ₅₀ (mg/ml)
<i>C. religiosa</i> bark	Lupeol	1.34 ±0.03
	Lupeol acetate	1.28 ±0.03
<i>S. grande</i> bark	Friedelin	1.91±0.03
	Betulinic acid	0.78±0.01
	Gallic acid	0.70±0.01
<i>C. communis</i>	b-sitosterol b-D-glucoside	1.79±0.03
	Stigmasterol	1.76±0.03
	Taraxasterol	0.47 ±0.01
<i>Spirulina</i>	Stearic acid	1.91±0.03
<i>G. procumbens</i> leaf	Vitamin C	1.80±0.03
	b-sitosterol	1.12 ±0.02
<i>M. charantia</i> fruit	Charatin	1.10 ±0.02
Voglibose (Reference)		0.32±0.005

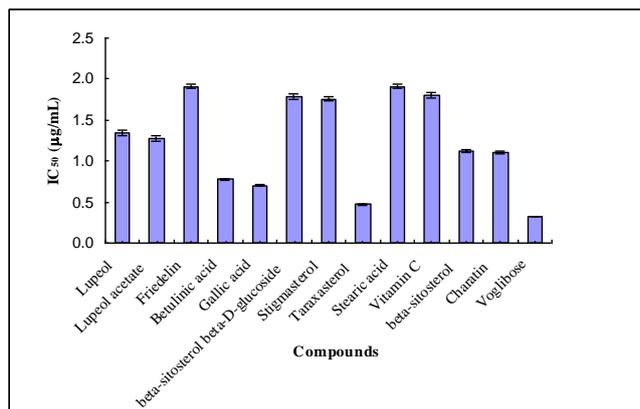


Figure 3.1 Comparison of α -glucosidase inhibitory effect of isolated compounds and reference drug Voglibose

4. Conclusion

All of the selected medicinal plants were generally found to possess α -glucosidase inhibitory effect and they may be useful for the formulation of α -glucosidase inhibitors to manage the Type 2 diabetes mellitus. Therefore, this research work is hoped to contribute to the development of Myanmar traditional medicinal formulation using plant sources for the treatment of postprandial hyperglycaemia, Type 2 diabetes, non-insulin dependent diabetes.

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