Chemical Analysis and $\alpha$-Glucosidase Inhibitory Effect of Bizat Leaves ($Eupatorium odoratum$ Linn.)
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Abstract
This research deals with the investigation of some chemical properties and the $\alpha$-glucosidase inhibitory effect of ethanol extract and watery extract from Bizat leaf ($Eupatorium odoratum$ Linn.). The Bizat leaf sample was collected from Pyay University campus. The 50% inhibitory concentration ($IC_{50}$) values of watery and ethanol extracts from Bizat leaves on $\alpha$-glucosidase enzyme activity were found to be 0.14 and 0.31 µg/mL, respectively, lower than that of standard metformin ($IC_{50}=0.42$ µg/mL) under conditions. Therefore, both watery and ethanol extracts from Bizat leaf were observed to be more effective than standard drug $\alpha$-glucosidase inhibitor metformin to inhibit the $\alpha$-glucosidase enzyme activity.

Key words: $\alpha$-glucosidase inhibitory effect, Bizat leaf, $Eupatorium odoratum$ Linn.

Introduction
Nowadays, people suffered from diabetes mellitus are rapidly increasing. It is also one of the six prior major diseases: diabetes, tuberculosis, hypertension, malaria, diarrhoea and dysentery recorded in Myanmar. Most of the people have tried to use herbal drugs for management or for the treatment of the diseases. There are two common types of diabetes: Type I diabetes (insulin-dependent diabetes) and Type II diabetes (non-insulin dependent diabetes). Postprandial hyperglycaemia plays an important role in the development of Type II diabetes mellitus. Control of postprandial hyperglycaemia is thought to be very important in the treatment of diabetes.

$\alpha$-Glucosidase and diabetes

$\alpha$-Glucosidase is one of glucosidases located in the brush-border surface membrane of intestinal cells and body fluids. As it is the key enzyme of carbohydrate digestion increasing the blood glucose level, it is
one of the facts to develop the Type II diabetes after a meal (Fallah. et al., 2008)

**α-Glucosidase inhibitors**

α-Glucosidase inhibitors act directly to reduce the sharp increases in glucose level that occur immediately following ingestion of a meal. By using α-Glucosidase inhibitors, postprandial hyperglycaemia could be controlled or managed (Cannel et al., 1987). α-Glucosidase inhibitors, a class of diabetes drugs, also known as "starch blockers" 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 (brand name Precose), miglitol (Glyset), voglibose (Volix and others) and emiglitate have been approved to use as antidiabetes drugs. Metformin (Glucophage and others) or sulfonylureas (Diabinese, DiaBeta, Glynase, Micronase, Glucotrol, Glucotrol XL, Amaryl) have been reported to be more effective at managing blood glucose (Website 1). Numbers of research on the investigation of the effective natural α-Glucosidase inhibitors from herbals with antidiabetic potential have been reported.

Testing the effect of herbs with anti-diabetic potential on α-Glucosidase is a common procedure in herbal research laboratories by using UV-visible spectroscopy. This bio-assay is useful to screen and to elucidate the mechanism of action of hypoglycaemic herbs.

Recently, in Myanmar *Eupatorium odoratum* Linn. (Bi-zat) flowering with purple colour is claimed as a medicinal plant to use for the treatment of Type II diabetes. It is widely distributed in Myanmar. It has been proposed as a traditionally useful medicinal plant for the treatment of various kinds of diseases such as skin disease, cancer and diabetes etc. Scientific investigation on treatment of boil, infected by *Stapylococcus aureus* has already been done using ethanol and pet-ether extracts and it was found that this plant has high potency for the treatment of skin disease (Nwe Thin Ni, 2004 a). It has also been found that it is flavonoid in its rich in leaves (Nwe Thin Ni, 2004 b). However, there is no scientific evidence that this plant can be used for the treatment of diabetes.

*Eupatorium odoratum* Linn.

Myanmar name – Bizat
Botanical name - *Eupatorium odoratum* Linn.
Family name - Asteraceae
English name – Triffid-weed
Part used - leaves

*Eupatorium odoratum* is distributed through India, Indochina and common in all tropical countries. In Myanmar, it can be widely found anywhere. It is (up to 9 feet) shrubby with rather large, lanceolate, leaf blades coarsely toothed, especially near the base on long leafless branches which are spreading from the axils. These flowers are white, flowering time from August to October in the Western Countries, whereas in Myanmar, it is from November to March (Wealth of India, 1952).

The plant of *Eupatorium odoratum* consists of terpenoids, steroids, flavonoids and tannins. According to the literature, steroids, terpenoids and 13 flavonoids such as 2-hydroxy-3,4,4′,5′,6′-pentamethoxy chalone, 4,2′-dihydroxy-4′,5′,6′-trimethoxy chalone, 2′-hydroxy-4,4′,5′,6′-tetramethoxy chalone, 6′-hydroxy-4,2′,3′,4′–tetramethoxy chalone, 4′-methoxy 5,7-dihydroxy flavonone, kaempferol, quercetin and kaempferol -4′–methyl ether, etc., are present in this plants.

**Application of Eupatorium odoratum** Linn.s

Indigenous cellulosic raw materials for the production of pulp, paper and board were obtained from *Eupatorium odoratum* Linn. The whole plant is reported to possess medicinal properties. Bitterness of the plant can cure blood clotting. A decoction of the leaves is used for lactogogue and it is valued in native medicine as a cure for malaria and as a cough remedy. Cancer is also found to be cured by the decoction of the leaves of this plant. Not only this property but also rejuvenation power restoration was found and practised by natives of Myanmar. The fresh juices of the leaves can cure fresh cuts very easily. It is like tincture iodine for the cuts. The part of leaves are used externally in traditional medicine as a wound healing, skin abscess, diuretic, cathartic, intermittent, fever, ulcers, bilious, catarrh and influenza.

The present research focused on some chemical analyses and on scientific study of the antidiabetic property of Bizat leaves via the investigation of the inhibitory effect on $\alpha$-glucosidase activity using ethanol extract and watery extract.

**Materials and Methods**

*Eupatorium odoratum* Linn. (Bizat) leaves were collected from Pyay University Campus in April, 2010. It was identified at Department of
Botany, University of Yangon. The collected Bizat leaves were air-dried at room temperature after cleaning. These dried leaves were then powdered and stored in air-tight glass bottle. 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".

**Some Chemical Analyses of Bizat Leaves**

Some nutritional values such as moisture, ash, fat, protein, crude fiber and carbohydrate contents of the sample were determined by appropriate reported methods (AOAC, 1990). The elements present in dried powdered samples were qualitatively determined by EDXRF (Energy Dispersive X-Ray Fluorescence) technique using Shimadzu EDX-700 spectrometer in Universities' Research Center, Yangon University. In order to find out the types of organic constituents present in the sample, preliminary phytochemical investigation: tests for alkaloids (Trease and Evans, 1980), α-amino acids (Marini-Bettolo et al., 1981), carbohydrates (Molish’s Test) (Shriner et al., 1980), cyanogenic glycosides (Trease and Evans, 1980), flavonoids (Robinson, 1983), glycosides (Marini-Bettolo et al., 1981), organic acids (Robinson, 1983), reducing sugars (Finar, 1973), saponins (Shriner et al., 1980), steroids, tannins and terpenoids (Tin Wa, 1972) was carried out according to the appropriate reported methods.

**Screening of Inhibitory Effect of Bizat Leaf on α - Glucosidase Enzyme Activity**

α-Glucosidase enzyme was extracted from imgerminated flint corn seed and this was used for screening of the inhibitory effect of Bizat leaf ethanol and watery extracts.

The effect of ethanol extract and watery extract from Bizat leaves 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 (Astumi et al., 1990). This experiment was done in triplicate for each sample solution. Absorbance values obtained were used to calculate percent inhibition and 50% inhibitory concentrations.

(i) **Preparation of test sample solution**

2 mg of each extract and 10 ml of distilled water were thoroughly mixed with vortex mixer. The mixture solution was filtered and the stock solution was obtained. Then the test sample solutions were prepared in
different concentrations: 0.125, 0.25, 0.5, 1.0, 2.0 µg/mL by serial dilution method.

(ii) Reagents required

(i) \( \alpha \) - glucosidase enzyme

10 mg dissolved in 100 ml distilled water.

(ii) Substrate – sucrose dissolved in distilled water (37 mM)

(iii) Glucose oxidase reagent (commercial kit)

Glucose oxidase in special buffer

(iv) 6% dimethyl sulfoxide (DMSO)

(iii) 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. Then the above mixture was incubated at 37 °C for 30 min and the reaction was stopped by immersing the test tube into a boiling water bath for 10 min and allowed to cool to room temperature. Absorbance for all solutions was measured by using a UV-7504 spectrophotometer at 505 nm. Antidiabetic drug metformin was used as a standard.

From the absorbance values, percent inhibition of the sample on \( \alpha \)-glucosidase enzyme activity and average percent inhibition on \( \alpha \)-glucosidase enzyme activity were calculated by using following equations (Kurihara et al., 1994).

\[
\text{\% inhibition} = \frac{A_c - A - A_b}{A_c} \times 100
\]
Where,

\[
\% \text{ Inhibition} = \frac{A_c - A}{A_c} \times 100
\]

\( A_c \) = 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 \( \alpha \)-glucosidase enzyme activity was calculated by Linear Progressive Excel Program.

**Results and Discussion**

In this section, the resultant data obtained from the preliminary phytochemical investigation, some chemical analyses and determination of \( \alpha \)-glucosidase inhibitory effect of watery and ethanol extracts from Bizat leaves will be discussed.

Preliminary phytochemical investigation indicated the presence of \( \alpha \)-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids in Bizat leaves. There is no cyanogenic glycosides found in this sample and it can be so inferred that Bizat leaves may be free from harmful effect due to the toxic property of cyanogenic glycosides. Chemical analyses revealed that the dried Bizat leaves contained 7.70% of moisture, 12.40 % of ash, 4.0 % of fats, 6.80% of fiber, 8.75% of proteins and 60.35 % of carbohydrates, based on dry weight.

**\( \alpha \)-Glucosidase inhibitory effect of Bizat leaves**

\( \alpha \) - Glucosidase enzyme can produce the glucose and fructose from sucrose by enzymatic hydrolysis.

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

Therefore, the presence or absence of \( \alpha \)-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 \( \alpha \) - glucosidase in the presence of the herbal extract, it can be inferred that the sample has the \( \alpha \)-glucosidase inhibitory effect, ie., it is an enzyme inhibitor. If the glucose is still formed from the
sucrose by \( \alpha \)-glucosidase enzyme in the presence of the herbal extract, the herbal may not possess the \( \alpha \)-glucosidase inhibitory effect. The principle of this method can be expressed as follows:

\[
\text{Sucrose} + \alpha - \text{Glucosidase enzyme} \rightarrow \begin{cases} 
\text{Glucose} & \text{produced} \\
\text{No Enzyme inhibition effect} & \text{(Not } \alpha - \text{Glucosidase enzyme inhibitor)}
\end{cases}
\]

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 produced hydrogen peroxide 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 (Yuhoo, 2004).

The reaction involved in the process can be shown as follows:

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Glucose peroxidase}} \text{H}_2\text{O}_2 + \text{Gluconic acid}
\]

\[
\text{H}_2\text{O}_2 + \text{HN} - \text{C} - \text{NH}_2 + \text{phenol} \xrightarrow{\text{POD}} \text{Red pigment}
\]

The absorbance of the red pigment will be increased with increasing amount of glucose. 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 watery and ethanol extracts of Bizat leaves. This observation indicated that the extracts has the inhibitory effect on the α-glucosidase enzyme activity. The absorbance values of red pigment formed from the glucose decreased after addition with crude extracts of Bizat.

From the absorbance values, the resultant percent inhibition effects of the corresponding crude extract and standard drugs in various concentrations (0.125, 0.25, 0.5, 1.0, 2.0 µg/mL) on α-glucosidase enzyme activity are shown in Table 1. It was also found that the percent inhibition of crude extract was increased with increasing the concentrations as illustrated in (Figure 1).

From the percent inhibition, the respective IC₅₀ values were calculated from the plot of percent inhibition vs different concentrations using Linear Progressive Excel Program and the results are tabulated in Table 1. Comparative study was also made on the IC₅₀ values for watery and ethanol extracts from Bizat leaves with standard drug metformin. Since the lower the IC₅₀ values the higher the inhibitory effect, the α-glucosidase inhibitory effect of Bizat watery extract (IC₅₀=0.14 µg/mL) was found to be highest, followed by EtOH extract (IC₅₀= 0.31 µg/mL) and then by standard drug metformin (IC₅₀ = 0.42 µg/mL) as described in Figure 2.

Table 1 Percent inhibition of crude extracts from Bizat leaves and standard metformin in various concentrations on α-glucosidase enzyme activity and the corresponding IC₅₀

<table>
<thead>
<tr>
<th>Sample</th>
<th>% inhibition in different concentrations (µg/mL)</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>23.70 47.21 57.95 69.40 73.25</td>
<td>0.31</td>
</tr>
<tr>
<td>Watery extract</td>
<td>48.02 63.73 72.95 79.43 85.41</td>
<td>0.14</td>
</tr>
<tr>
<td>Metformin</td>
<td>38.85 45.25 51.85 58.29 74.07</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Figure 1  A plot of percent inhibition on $\alpha$-glucosidase vs different concentrations of Bizat leaves extracts and standard metformin

Figure 2  Comparison of the IC$_{50}$ values of watery and ethanol extracts from Bizat leaves with standard drug metformin
Conclusion

From the overall assessment of the present research work, the following inferences could be deduced.

Preliminary phytochemical tests revealed the presence of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids in Bizat leaves. In addition, Bi-zat leaves may be free from toxic effect due to the absence of the harmful cyanogenic glycosides.

The Bizat leaves contained 7.70% of moisture, 12.40% of ash, 4.0% of fats, 6.80% of fibre, 8.75% of proteins and 60.35% of carbohydrates, based on dry weight.

The watery extract (IC$_{50}$ = 0.14 µg/mL) and EtOH extract (IC$_{50}$ = 0.31 µg/mL) from Bizat leaves possessed the inhibition effect on α-glucosidase enzyme activity and these extracts were found to be more effective to inhibit the α-glucosidase enzyme activity than standard drug metformin (IC$_{50}$ = 0.42 µg/mL). This observation indirectly shows that Eupatorium odoratum Linn. (Bizat) leaves may possess the antidiabetes activity and may be effectively used as a natural α-glucosidase inhibitor or may be useful in the formulation of antidiabetes drugs to control or to manage the Type II diabetes mellitus.

This research will contribute to develop the role of formulation of antidiabetes drugs from medicinal plants to control or to manage the Type II diabetes mellitus.

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**Online Material**