Bitterness Potency and α–Amylase Inhibitory Activity of *Andrographis paniculata* Nees. (Say-ga-gyi)

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Abstract

This research aims at the biological investigations of Andrographis paniculata Nees. (Say-ga-gyi, SGG). Bitterness potency of water extract of selected plant was studied by WHO standard method using quinine hydrochloride as a standard. Bitterness value was determined to be 366 (SGG).These tested sample was found to be less bitter than standard quinine hydrochloride (bitterness value, 2000). The ethanol and water extracts of selected plant was also explored for the in vitro α–amylase inhibition and their activity was compared with standard anti-diabetic drug, acarbose. The IC50 values of ethanol and water extracts of the selected plant was observed to be 133.57 µg/mL, and 79.37µg/mL, respectively. The selected sample exhibited more potent α–amylase inhibition activity than acarbose (IC_{50} 200.92 µg/mL). The resulted data suggested that Andrographis paniculata Nees. (Say-ga-gyi, SGG) is an important medicinal plant for the treatment of diabetic and stress related disease.

Keywords:*Andrographis paniculata* Nees. , bitterness potency, α-amylase inhibition activity

Introduction

Myanmar has a long history of medical tradition and traditional learning of plants remedies for many diseases including diabetes mellitus, cancer, malaria and tuberculosis persist until now. Myanmar traditional and Ayurvedic physicians successfully cure or control many diseases, through herbal medicine. There are many medicinal plants which have been used in Myanmar traditional medicinal formulations (TMF) which possess six different tastes such as sweet, sour, salty, pungent, bitter and astringent. Medicinal plants materials that have a strong bitter taste are employed therapeutically as appetizing, antidiabetic, anticancer and antimalarial agents. At present, the Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in combating six major types of disease: malaria, tuberculosis, diarrhea, dysentery, diabetes and hypertension. So, Department of Chemistry, University of Yangon, Myanmar has studied the chemical constituents and pharmacological activities of Myanmar indigenous medicinal plants and Myanmar traditional medicinal formulation (TMF).

There are many bitter traditional medicinal plants used for the treatment of diabetes mellitus and diseases related to the oxidative stress. Among them, *Andrographis paniculata* Nees. (Say-ga-gyi, SGG) was selected for Bitterness Potency and α–Amylase Inhibitory Activity investigations in the present study. This bitter traditional medicinal plants has been used as antidiabetic plant in Myanmar traditional system but there is no systematic and scientific evaluation. Thus, the present study intended to illustrate the scientific proof of Myanmar medicinal plants to be used as good remedies in the treatment of diabetes mellitus.

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Botanical Aspect of *Andrographis paniculata* Nees. (Say-ga-gyi, SGG)

Family	:	Acanthaceae
Botanical name	:	Andrographis paniculata Nees
English name	:	king of bitters, andrographis
Myanmar name	:	Say-ga-gyi
Part used	:	Leaf, stemand Root

Uses of *Andrographis paniculata* Nees. (Say-ga-gyi, SGG)

Andrographis paniculata Nees. is an important medicinal plant and widely used around the world. In recent times, commercial preparations of this plant extracts are also used in certain countries. However, the preparations yet need to be standardized for their better efficacy. The aerial part of *Andrographis paniculata* is most commonly used; its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe. *Andrographis paniculata* has been reported to have a broad range of pharmacological effects including anticancer, antidiarrheal, antihepatitis, anti-HIV, anti-hyperglycemic, anti-inflammatory, antimicrobial, antimalarial, antioxidant, cardiovascular, cytotoxic, hepatoprotective, immunostimulatory, and sexual dysfunctions. (Kook, *et al*., 2009). Photographs of *Andrographis paniculata*Nees. are as shown in figure 1.

(a) Whole plant (b) Dry plant(c) Dry Powder

Figure 1.Photographs of *Andrographis paniculata Nees. (Say-ga-gyi, SGG)*

Materials and Methods

Reagents and Equipment

Distilled water and ethanol (used for the preparation of extracts), quinine hydrochloride (used as standard for bitterness value determination) and standard acarbose, starch, I_2, HCL (used for α-amylase inhibition assay) were analytical grade. The enzyme, α-amylase was directly obtained from the human saliva. A shaker, water-bath and electric stove were used for the extractions and a UV-Vis Spectrophotometer (UV-7504, KWF, China & UV-1800, Shimadzu, Japan) was employed for the spectrophotometric determinations. Linear excel program was performed for the calculation.

Preliminary Phytochemical Test

A few gram of dried powder of selected sample was subjected to the tests of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, tannins, steroids, terpenoids according to the standard procedures. (Trease and Evans, 1980, Robinson, 1983, M-Tin Wa, 1970, Volgel, 1996, Harborne, 1984, Marini-Bettolo *et al.*, 1981)

Determination of Bitterness Value

The bitterness value is the reciprocal of the dilution of a compound, a liquid or an extract that still has a bitter taste. Medicinal plant materials have a strong bitter taste act as appetizing agents. The bitter properties of plant materials are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.

Preparation of Solution

(i) Preparation of Stock and Diluted Standard Quinine Solution

Accurately weighed 0.1 g quinine hydrochloride (R) was dissolved in safe drinking water to produce 100 mL. This solution (1 mL) was further diluted to 100 mL with safe drinking water. This stock solution of quinine hydrochloride (SQ) contained 0.01 mg/mL. Nine serial dilutions were made each containing 0.042, 0.044, 0.046, 0.048, 0.050, 0.052 0.054, 0.0.54 and 0.058 mL solution of CQ and volume made up to 10 ml with safe drinking water and obtaining a concentration of 0.040, 0.042, 0.044 up to 0.056 mg/10mL (indicate in Table 1).

Table 1. Determination of Bitterness Value; Serial Dilution for Initial Test (Quinine Hydrochloride)

Test tube No.	1	2	3	4	5	6	7	8	9
V_Q/mL	4	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6
V_W/mL	6	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4
C_Q (mg/10 mL)	0.040	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056

V_Q = volume of quinine hydrochloride V_W = volume of water

C_Q = Concentration of quinine hydrochloride

(ii) Preparation of Stock and diluted solutions of the plant material

The stock solution (1 mg/mL) of each extract was prepared by dissolving 1 mg of water extract from section 2.3.4 in 1 mL of distilled water (C_s). Nine serial dilutions of each water extract were prepared as shown in Tables 2.

Table 2 Serial Dilution for the Determination of Bitterness Potency of SGG

Test tube No.	1	2	3	4	5	6	7	8	9
V_s/mL	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
V_W/mL	9.0	8.5	8.0	7.5	7.0	6.5	6.0	5.5	5.0

V_S = volume of sample V_W = volume of water

Procedure

After rinsing the mouth with safe drinking water, 10 mL of the most dilute solution was tasted while swirling it in the mouth mainly near the base of tongue for 30 s. After 30 s the solution was spit out and it was ascertained for 1 min whether a delayed sensation for bitterness existed. Then mouth was rinsed with safe drinking water. The next highest concentration was not tasted until at least 10 min. The lowest concentration at which material continues to provoke a bitter sensation after 30 s was referred the threshold bitter concentration. After a first series of test, rinsed the mouth thoroughly with safe drinking water until no bitter sensation remains, wait at least 10 min before carrying out the second test.

In order to save time in the second test, it was advisable to ascertain first whether the solution in tube no.5 gave a bitter sensation. The threshold bitter concentration of the material was found by tasting the dilutions in tubes 1-4. If the solution in tube no.5 did not give a bitter sensation, the threshold bitter concentration was found the dilutions in tubes 6-10.

All solution and the safe distilled water for mouth washing should be at 20-25°C.

The bitterness value in units per g was calculated by using the following formula.

$$\text{Bitterness value (unit/g)} = \frac{2000 \times C}{A \times B}$$

Where

A = Concentration of stock solution (C_s) mg/mL

B = Volume of (V_s) ml tube with threshold bitter concentration

C = Quantity of quinine hydrochloride (in mg) tube with threshold bitter concentration.

Determination of α-Amylase Inhibition Potency

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitory activity was based on the starch iodine method thatwas originally developed by Fuwa (1954) and later employed by others for determination of amylase activity in plant extracts with some modifications.

Preparation of Solutions

(i) Preparation of α-Amylase Enzyme Solution

First, 1 mL of saliva from a volunteer was collected in a small beaker (25 mL) and then it was diluted with 9 mL of deionized water.

(ii) Preparation of Stock and Diluted Solutions of Test Samples

3 mg of tested samples (ethanol extract, water extract and acarbose (standard) were dissolve in small amount of methanol and the volume was made up to 3 mL with 0.02M phosphate buffer solution (pH 6.7) in small beaker (25 mL). Stock solution of tested samples at 1000 μg/mL concentration was obtained. This stock solution was serially two-fold diluted with 0.02 M phosphate buffer solution (pH 6.7) to get the concentration of 1000, 500, 250, 125 and 62.5 μg/mL.

Procedure

In alpha amylase assay, the starch-iodine was used. First 2 mL of (0.5%) substrate starch solution and 1 mL of tested solution (Acarbose standard drug, ethanol extract and aqueous extract) of five different concentration such as 62.5, 125, 250, 500, and 1000 μg/mLwere added in a bottle and these mixture was incubated for 3 min at room temperature. To start the reaction, 1 mL of α-amylase was added in above solution followed by incubated for 15 min at room temperature. To stop the reaction, 4 mL of 0.1M HCl was added in this mixture and to detect the reaction, 1 mL of Iodine-iodide indicator (1 mM) was added in the mixture. Absorbance was read at 650 nm by UV spectrophotometer in the visible region. The control solution was prepared as above procedure by using phosphate buffer (0.02M, pH 6.5) instead of drug solution.

All the experiments were done in triplicate. Percent inhibition of each sample solution was calculated by using the following formula. Standard deviation (SD) and 50% inhibition concentration (IC_{50}) value in μg/mL were calculated by computer excel program.

$$\% \text{ Inhibition} = \frac{A_{Sample} - A_{Control}}{A_{Sample}} \times 100$$

Where,

$A_{control}$ = the absorbance of the control solution

A_{sample} = the absorbance of sample solution

Results and Discussion

The phytochemical tests revealed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acid, phenolic compounds, reducing sugars, saponins, steroid, tannins and terpenoids were found to be present but and starch was absent in selected sample.

The World Health Organization (WHO) prescribed a number of quality control tests that medicinal plant material should undergo (WHO, 1998). Determination of bitterness by sensation is subjective and cumbersome, and does not readily appeal to every analyst. However, determination of bitterness by taste, as in the present study, remains an appropriate method. It is well known that medicinal

plant materials called “bitters” are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice. The bitterness of selected plants were determined by the method described by WHO which compares the threshold bitter concentration of an extract of the herb with the threshold bitter concentration of a dilute solution of quinine hydrochloride. According to the result, it is shown in Table 3.

Table 3. Result of Bitterness Values of Selected Sample and Standard (Quinine hydrochloride)

Sample	Bitterness Value
SGG	366
Quinine Hydrochloride	2000

Hyperglycemia has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of macro and microvascular complications. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including *α*-glucosidase and *α*-amylase present in the brush borders of intestine.In this study, the *α*-amylase inhibitory activity of *A. paniculata* (Say-ga-gyi, SGG) was investigated. The inhibitory effect of ethanol extracts and water extracts were analyzed. The percentage inhibition of *α*-amylase by ethanol and water extracts were studied in a concentration range of 6.25-1000 *μ*g/ mL. The percentage inhibition of the samples on *α*-amylase enzyme activity increased with increasing the concentrations.From the percentage inhibition, the respective IC_{50} values for the plant extracts were calculated and the results are respectively tabulated in Tables (4 and 5). The ethanol and water extract of bitter selected plant was also explored for the in *vitroα*-amylase inhibition and their activity was compared with standard anti-diabetic drug, acarbose. The 50% a–amylase inhibition potency (IC_{50}) of ethanol and water extracts of the selected plant was ranging between 79.37-133.57 mg/mL, indicating that crude extracts possessed potent a–amylase inhibition activity than standard acarbose (IC_{50} 200.92 mg/mL). These observations are dedicated with a bar graph diagram in Figures 2,3 and 4.

Table 4. α-Amylase Inhibition Activity of Ethanol Extract of SGG and Acarbose

Sample	%Inhibition (mean ± SD) in different concentration (μg/mL)					IC_{50} (μg/mL)
	62.5	125	250	500	1000	
SGG	34.12 ± 2.52	49.34 ± 2.39	59.88 ± 1.80	68.37 ± 0.70	83.07 ± 0.30	133.57
Acarbose	24.87 ±	31.61 ±	62.05 ±	87.25 ±	92.75 ±	200.92

	3.90	3.64	0.76	0.11	0.05

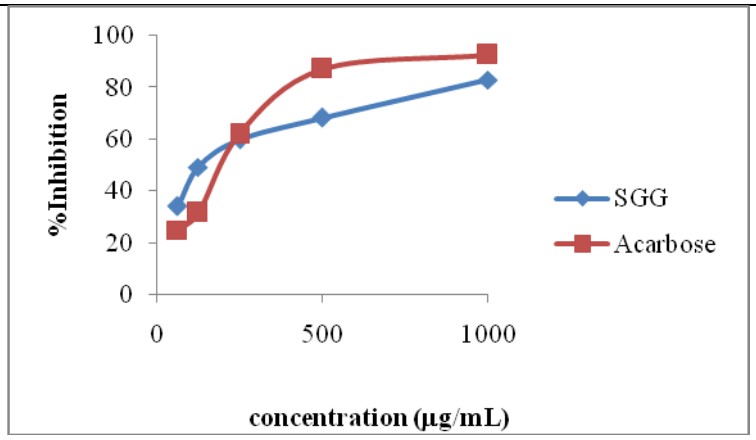

Figure 2. A plot of α-amylase inhibition activity of ethanol extract of SGG and Acarbose

Table 5. α-Amylase Inhibition Activity of Water Extract of SGG and Acarbose

Samples	% Inhibition (mean ± SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	62.5	125	250	500	1000	
SGG	42.33 ± 2.73	70.92 ± 1.11	79.84 ± 0.48	85.79 ± 0.14	86.69 ± 0.18	79.37
Acarbose	24.87 ± 3.90	31.61 ± 3.64	62.05 ± 0.76	87.25 ± 0.11	92.75 ± 0.05	200.92

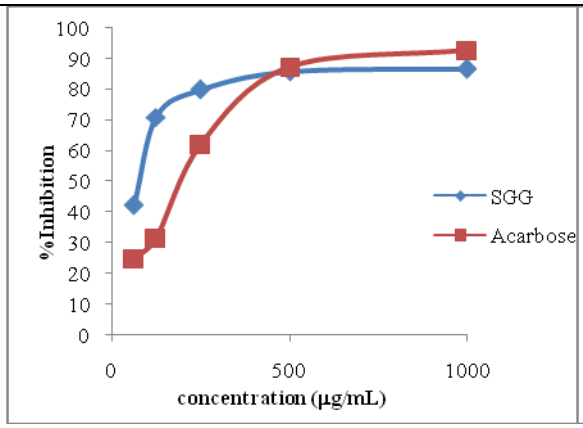

Figure 3. A plot of α-amylase inhibition activity of water extract of SGG and Acarbose

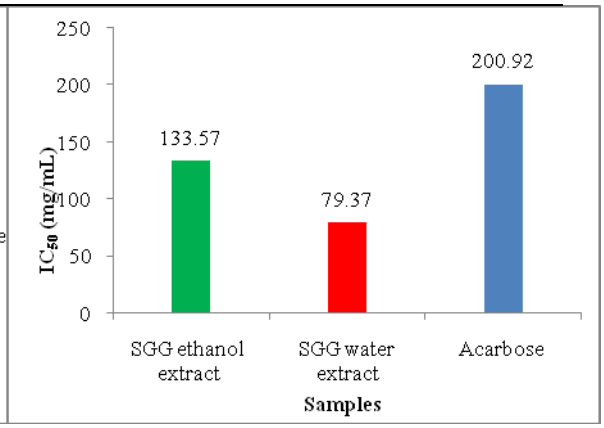

Figure 4. A bar graph of IC_{50} µg/mL of different extract of SGG and Acarbose

Conclusion

From the overall assessment of the Bitterness Potency and α–Amylase Inhibitory Activity of *Andrographis paniculata* Nees. (Say-ga-gyi, SGG)the following inferences may be deduced.

From the preliminary phytochemical investigation, it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids were present in the selected samples. Whereas, starch was not detected in selected bitter Myanma medicinal plants.

Bitterness potency of water extract of each plant was studied by WHO standard method using quinine hydrochloride as a standard. Bitterness values were determined to be 366 (SGG). The tested samplewas found to be less bitter than standard quinine hydrochloride (bitterness value, 2000).

The ethanol and water extracts of bitter selected plantwas also explored for the *in vitro* α–amylase inhibition and their activity wascompared with standard anti-diabetic drug, acarbose. The 50% α–amylase inhibition potency (IC_{50}) of ethanol and water extracts of the selected plantwas ranging between 79.37–133.57 μg/mL, indicating that crude extracts possessed potent α–amylase inhibition activity than standard acarbose (IC_{50} 200.92 μg/mL).

The current study demonstrated that *Andrographis paniculata* Nees. (Say-ga-gyi, SGG) is important medicinal plants. Thesepossesses are not only phytochemicals but also they exhibited promising *in vitro* α–amylase inhibition activity thereby proving to be potential hyperglycemic agents.

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