

Comparative Studies on Phytochemical Screening and Blood Glucose Lowering Activity of Leaves of *Gynura procumbens* (Lour.) Merr and *Ocimum americanum* L.

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Abstract

Indigenous medicines which have been derived either directly or indirectly from plants have been used for treatment of diabetes in several countries including Myanmar. Consequently, Myanmar indigenous medicinal plants (*Gynura procumbens* and *Ocimum americanum*) which are used to lower the blood glucose lowering activity. The selected plants were screened for phytoconstituents by test tube method. The anti-diabetic activity of plant samples were investigated by chemical method. The present work aims to determine the *in-vitro* blood glucose lowering ability of selected plants which are commonly used as antidiabetic drug. From the phytochemical screening results showed that flavonoids, alkanoids, terpenoids, tannins, saponins, steroids were present in *Gynura procumbens* and flavonoid, alkanoids, steroid, tannins were present in *Ocimum americanum*. From the experimental results, the effect of lowering glucose activities of *Ocimum americanum* is higher than that of *Gynura procumbens*.

Keywords: nutritional value, minerals, phenolic compounds, antidiabetic

Introduction

Diabetes, the silent killer is an extremely dangerous health disorder from which many people suffer these days. This disease can affect various organs of the body as well as lead to other serious ailments. One of the causes of diabetes is the lack of adequate insulin production in the body and is called Type I. Another is caused by insulin resistance and is termed Type II. Both types are associated with short and long-term complications that affect the individual's quality of life and often engender fear and powerlessness and compromise physical and psychological functioning. Some common symptoms of diabetes are sudden weight loss, thirst and hunger, lack of vision, excessive and cuts, bruises or wounds taking a long time to heal (Kumar, 2004).

This deadly disease should not neglect at any cost and should be treated as soon as it is detected as it can lead to various diseases of kidney and other organs or can even lead to permanent blindness (American Diabetes Association, 2014).

Material and Method

Samples Collection

Two leaves, (Pinzein and Pyar-mee-swè) well-known as anti-diabetic medicines were collected as the samples. The leaves were collected from Myingyan Township, Mandalay Region.

Leaves

Myanmar Name : Pinzein

English Name : Tulasi

Botanical Name : *Ocimum americanum* L.

Family : Labiatae (Lamiaceae)

Part Used : Leaves



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Myanmar Name : Pyar-mee-swe'
 English Name : -
 Botanical Name : *Gynura procumbens* (Lour.) Merr
 Family : Compositae, Asteraceae
 Part Used : Leaves



Procedure

(a) Preparation of Sample Solutions

For *O. americanum*, the fresh leaves were used as samples. 20 g sample was weighed, boiled with distilled water for 30 seconds, filtered, centrifuged the filtrate and the volumes of sample solution were determined.

For *G. procumbens*, 20 g of each fresh leaves were weighed and preparation of water extract was made as above respectively (*Molecular Aspects of Medicine*, 2006).

(b) Preparation of Standard Glucose Solution

Exactly 18 mL of 5 g/20 mL glucose injection was measured and placed in a 100 mL volumetric flask. Then, it was diluted with distilled water up to the mark of the flask (Bennhoff, 2010).

(c) Preparation of Fehling's Solution

Exactly 3.465 g of copper (II) sulphate was weighed and dissolved with distilled water to obtain 50 mL of Fehling's solution A.

Exactly 17.30 g of potassium sodium tartrate and 12.5 g of potassium hydroxide were weighed and dissolved together with distilled water to obtain 50 mL of Fehling's solution B.

Just before the experiment, the equal volume of freshly prepared Fehling's solution A and B were mixed thoroughly and the resulting Fehling's solution was diluted with an equal volume of distilled water (*J. Metabol. Res*, 1923).

(d) Standardization of Fehling's Solution

Freshly prepared 50 mL of Fehling's solution A and 50 mL of Fehling's solution B were mixed in a reagent bottle and then 100 mL of distilled water was added into it and shaken thoroughly to obtain 200 mL of Fehling's solution.

20 mL of Fehling's solution was taken out and placed in a conical flask. Three drops of methylene blue indicator were added into it and the solution was heated up to about 90°C. Then, the heated Fehling's solution was titrated with standard glucose solution cautiously by adding drop-by-drop until the deep blue color of Fehling's solution has completely disappeared. This process was repeated three times.

From the resulting titre, concentration of standard glucose solution and reaction stoichiometry, the concentration of Fehling's solution was calculated.

In order to confirm the methylene blue end point, when the blue colour of Fehling's solution has completely disappeared (the end point was reached), the solution was filtered to remove the red Cu₂O, precipitate and the filtrate was heated and standard glucose solution was added into the heated filtrate solution and observation was made (*J. Metabol. Res*, 1923).

(e) Determination of the *In-Vitro* Ability of Sample Solution for Lowering the Glucose

15 mL of sample solution (watery extract) was mixed with 15 mL of standard glucose solution and shaken thoroughly to be homogeneous. The solution was stood for 2 hours.

Then, 20 mL of Fehling's solution was taken out and placed in a conical flask. Three drops of methylene blue indicator were added into it and was heated up to about 90 °C. The heated Fehling's solution was titrated cautiously with homogeneous solution mixture of glucose and sample solution until deep blue color of Fehling's solution has completely disappeared. The process was repeated for three times.

From the concentration of Fehling's solution, titre volume of glucose and sample mixture solution and reaction stoichiometry, the concentration of glucose in the mixture solution was calculated.

Finally, decreased amount of glucose in 2 hours duration by unit weight of sample was calculated (*J. Metabol. Res*, 1923).

(f) Determination of the Most Effective Time Duration for Lowering the Glucose

When the sample solution is obtained, equal volumes of sample solution and standard glucose solution were mixed quickly and time was noted. Starting from 15 minutes after mixing, 20 mL of standardized Fehling's solution was titrated with mixture solution of standard glucose and sample solution. The procedure was repeated at the same interval of time as 30 min, 45 min, 60 min, ... till there is no change or nearly no change in concentration of glucose in mixture solution (Bennhoff, 2010).

Results and Discussion

Results for the Preparation of Sample Solutions

From the two selected samples, which are said to be anti-diabetic herbs, the water extracts were prepared.

Table (1) Data for the Preparation of Sample Solution

No.	Sample	Weight of sample	Volume
1.	<i>Ocimum americanum</i> L. Pinzein	20 g	48.0 mL
2.	<i>Gynura procumbens</i> (Lour) Merr Pyar-mee-swè	20 g	39.6 mL

According to these results, watery extract of leaves of *Ocimum canum* Sims is the more effective and 1 g of fresh sample causes to decrease 0.4944 mmol or 88.99 mg of glucose by using its watery extract. Similarly, the watery extract of *Gynura procumbens* (Lour.) Merr is also effective and 1 g of fresh sample causes to decrease 0.406 mmol or 73.08 mg of glucose by using its water extract.

Table (2) Experimental Data and Results for Amount of Glucose

No	Sample solution	W (g)	V (mL)	V _{titre} (mL)	M _f (M)	Decrease of glucose (mmol g ⁻¹)
1	<i>Ocimum americanum</i> L.	20	48.0	17	0.0220	0.4944
2	<i>Gynura procumbens</i> (Lour.) Merr	20	39.6	16.7	0.0224	0.4060

According to the resulting data, it can be seen obviously that the most effective time duration for lowering the glucose is found to be at least 2 hours' duration.

Table (3) The Results of Titre (V_{titre}) Concentration of Glucose at Time Interval t (M_t) and Decreased Concentration of Glucose at Time Interval t (M_{dec})

No	Time interval (min)	Watery extract of <i>Gynura procumbens</i> (Lour.) Merr			Watery extract of <i>Ocimum americanum</i> L.		
		V_{titre} (mL)	M_t (mmol mL ⁻¹)	M_{dec} (mmol mL ⁻¹)	V_{titre} (mL)	M_t (mmol mL ⁻¹)	M_{dec} (mmol mL ⁻¹)
1.	15	5.0	0.0750	0.0500	5.1	0.0735	0.0515
2.	30	5.1	0.0735	0.0515	5.3	0.0707	0.0543
3.	45	5.3	0.0707	0.0543	5.5	0.0681	0.0569
4.	60	5.5	0.0681	0.0569	5.9	0.0635	0.0615
5.	75	5.8	0.0646	0.0604	6.5	0.0576	0.0674
6.	90	6.1	0.0614	0.0636	6.9	0.0543	0.0707
7.	105	6.5	0.0576	0.0674	7.3	0.0513	0.0737
8.	120	6.6	0.0568	0.0682	7.9	0.0506	0.0744

Table (4) The Results of Phytochemical Test for Pyar-mee-swe' and Pinzein Leaf

No.	Constituent	Reagent used	Observation	Pyar-mee-swe'	Pinzein
1.	Flavonoids	Ethanol, conc: HCl, Mg turnings	No red color solution	+	+
2.	Alkanoids	Wagner's reagent Dragendroff's reagent	Brown ppt: Orange ppt:	+	+
3.	Terpenoids	Ethanol, CHCl ₃ , conc: H ₂ SO ₄	No pink color solution	+	-
4.	Tannins	H ₂ O, 10 % FeCl ₃ , dil:H ₂ SO ₄	Yellowish brown ppt	+	+
5.	Saponins	H ₂ O, vigorously shaken (3 mins)	Froth	+	-
6.	Steroids	Ethanol, 1 mL of acetic anhydride, conc: H ₂ SO ₄ , CHCl ₃	Green color solution	+	+

(+) = present, (-) = absence

Conclusion

In this research work, two kinds of sample, *Ocimum americanum* L. (Pinzein) and *Gynura procumbens* (Lour.) Merr (Pyar-mee-swe') were selected as samples.

All these two samples are well-known and said to be used for anti-diabetic herbs. Hence, in order for these samples to observe actually effective or not. The *in-vitro* ability of these samples for lowering the glucose was determined. According to the physicians, as the efficacy of medicine or something eaten to be shown effectively require for at least 2 hours,

the *in-vitro* ability of sample herbs for lowering the glucose had investigated at 2 hours duration after mixing the sample solutions with glucose.

The experimental results showed that the two selected samples could be observed to have the *in-vitro* ability for lowering glucose. The watery extract of *Ocimum americanum* L. (Pinzein) and *Gynura procumbens* (Lour.) Merr (Pyar-mee-swe') were found to be the effective with the ability of lowering glucose 0.4944 mmol g⁻¹ and 0.4060 mmol g⁻¹ respectively.

The minimum time duration for the effective ability of lowering the glucose was investigated by using two effective sample solutions. The results showed that the minimum time duration for the effective ability of lowering glucose is at least 2 hours for these two-sample solution.

According to this research work, the two selected samples could be observed to be suitable to use as the anti-diabetic medicine or anti-diabetic food. Among them, the watery extract of *Gynura procumbens* (Lour.) Merr (Pyar-mee-swe') should be used to lower the great amount of glucose within 2 hours and the watery extract of *Ocimum americanum* L. (Pinzein) should be used to lower the glucose quickly.

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