Screening of Alpha- Glucosidase Inhibitory Effect of Fruits of *Terminalia chebula* Retz. (Phan- khar)

Aye Mi Mi Htwe*

Abstract

This research deals with the preliminary phytochemical investigation and α- glucosidase inhibitory effect of fruit of *Terminalia chebula* Retz. (Phan-khar). In the fruits of *Terminalia chebula* Retz. (Phan-khar), α- amino acids, glycosides, phenolic compounds, steroids, terpenoids, tannins, reducing sugars, saponins, and organic acids were found to be present according to preliminary phytochemical tests. However, alkaloids, carbohydrates, cyanogenic glycosides, flavonoids, and starch were not detected in the fruit of *Terminilia chebula* Retz. (Phan- khar). The α- glucosidase inhibitory effect of watery and 95 % ethanol extracts were determined. α- glucosidase inhibitory assay method revealed that IC_{50} of watery extract and 95 % ethanol extract were 0.52 µg/ mL and 0.43 µg/ mL, respectively. Test results revealed that ethanol extract was more effective than watery extract. Standard Voglibose, synthetic medicine was used as a standard reference which showed IC_{50} of 0.32 µg/ mL. Test sample of *Terminilia chebula* Retz. (Phan- khar) fruits showed mild activity when compared to synthetic medicine. However, it is hope that the *Terminilia chebula* Retz. (Phan- khar) fruits can be used as α-glucosidase inhibitor for treatment of diabetes as a natural product.

Keywords: *Terminilia chebula* Retz., phytochemical constituents, α-glucosidase inhibitory activity

Introduction

In recent years, much interest has been focused on biologically active compounds occurring in natural resources. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Plant based drugs have been in use against various diseases since time immemorial. The essential values of some plants have long been published but a large number of them remain unexplored as yet. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues.

Medicinal plants are the backbone of traditional medicine. Traditional medicine has been practiced in our country for centuries. Myanmar has a rich in variety of medicinal plants and people use various herbal medicine. But so far, there has not been full and systematic exploitation of this natural resource (May Aye Than 2002).

α-glucosidase is an important enzyme on dietary carbohydrate digestion and post-translational processing of glycoproteins. Small intestinal alpha-glucosidase hydrolyzes carbohydrate to glucose, which is absorbed through the gut wall to become the blood glucose and to cause postprandial hyperglycemia. It has been recognized that alpha-glucosidase inhibitors can prevent against some diseases e.g. diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia and have anti-tumor and anti-HIV activity. Many research works were focused on microorganisms such as actionmycete, microalgae, cyanobacteria (Xiao, and Rongli, 2005).

α-glucosidases widely occurred in plant tissues, seeds, fruits, leaves and roots. The seeds of rice, flint corn, buckwheat, millet, barley, sugar beet, malted barley and almond, the seedling of alfafa, pea and soyabean, potato (tuber), carrot (root), tomato (fruit) and lupin (leaves and stems) contain a relatively large amount of α-glucosidase.

*Dr, Professor, Department of Chemistry, Magway University

α-glucosidase is distributed in mammalian tissues, animal tissues and body fluids. Human urine, intestinal cell, pig intestinal mucosa, livers of dog, cattle and rat, kidney of human and rat, rabbit and cow, plasma of human, horse, cattle, sheep, dog, cat, rabbit, swine, guinea pig and rat contain a large amount of α- glucosidase.

***Terminalia chebula (*T. *chebula)* Retz.**

*Terminalia chebula (*T. *chebula)* Retz (Combretaceae) is a native plant in India and Southeast Asia. Its dried ripe fruits, has traditionally been used to treat various ailments in Asia. The ability of T. *chebula* fruit extract in significantly increasing the body weight and effectively controlling the increase in blood glucose levels in diabetic group of rats may be attributed to its antihyperglycemic effects. The observed increase in the level of plasma insulin indicates that T. *chebula* fruit extract stimulates insulin secretion from the remnant beta-cells or from regenerated beta-cells. In this context, the plant has also been reported to exert hypoglycemic activity through insulin release stimulatory effect (Pari and Latha, (2002), Chattopadhyay, (1999)).

Botanical aspect of *Terminalia chebula* Retz (Phan-khar)

Botanical Name : *Terminalia chebula* Retz
Family : Combretaceae
English Name : Leaf-gall, Myrobalan
Myanmar Name : Phan-khar
Plant Parts Used : leaf, bark, fruit, seed

Uses of Phan- khar (*Terminalia chebula* Retz.)

Terminalia chebula Retz.is a traditional medicine belonging to the genus Terminalia, family Combretaceae. The dried ripe fruit of T.*chebula* is an important India herb used extensively in the indigenous system of medicine for its homeostatic, antitussiue, laxative, diuretic, and cardiotonic activities. A fruit, finely powdered, as used as dentifrice. They are also useful in the treatment of asthma, piles, diabetes and cough and have antibacterial and antioxidant properties. They are used as gargle against inflammation of mucous membrane of mouth (Sabu, 2002).

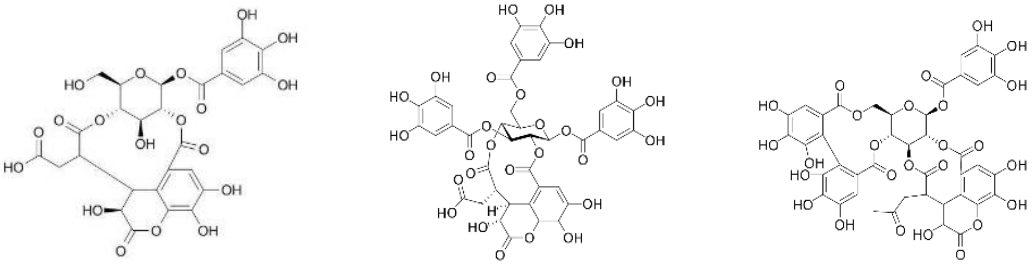
(a) Fresh fruit of Phan-khar

(b) Nature of Phan-khar

Figure 1. Photographs of *Terminalia chebula* Retz. (Phan-khar) fruit

Chemical constituents of Phan-khar

In Phan-khar fruits, Chebulanin, Chebulinic acid, and chebulagic acid to be present (Saleem, 2002). Structures of some chemical constituents in Phan-Khar fruits are described in Figure 2.



(a) Chebulanin (Riaz, 2017) (b) Chebulinic acid (c) chebulagic acid

Figure 2. Some chemical constituents in Phan-khar fruits

Materials and Methods

Sample Collection and Preparation of Plants Materials

The medicinal plant of Phan-khar (*Terminalia chebula* Retz) fruits was chosen to be studies in the present research. The sample of Phan-khar (*Terminalia chebula* Retz) fruits were collected from Magway Township, Magway Region. The collected fruit samples were cleaned by washing thoroughly with water and air-dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. These powered samples were labeled and separately stored in air-tight containers.

Preliminary Phytochemical Test

Dried powder of Phan-khar fruits 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, 1972, Volgel, 1998, Harborne, 1998, Marini *et al.,* 1981)

Extraction of Crude Extracts from the Fruits of Phan- khar

Procedure

Dried powdered sample of Phan- khar (*Terminalia chebula* Retz.) fruits (100 g) were defatted with petroleum ether (60- 80 °C, 300 mL) for 48 hours (2 days). It was then filtered and the filtrate was discarded. Petroleum ether solution was recovered by distillation and evaporation to dryness to give petroleum ether extract (0.2 g, 0.2 %). The residue was extracted with 95 % ethanol 300 mL for 72 hours (3 days) and then filtered.

95 % ethanol solution was recovered by distillation and evaporation to dryness to give 95 % ethanol extract (31.0 g, 31.0 %).

To prepare the water extract, the dried powdered sample of Phan- khar (*Terminalia chebula* Retz.) fruits (100 g) were extracted with distilled water (200 mL) for 2 hours, by using hot extraction. Water solution was removed by exasperation to dryness to give water extract (21.0 g, 21.0 %).

Screening of α-glucosidase inhibition activity of crude extracts from Phan-khar fruits by α-glucosidase inhibition assay

(a) Isolation and purification of α-glucosidase from flint corn seeds sample

Flint corn seeds (orange colour) were purchased from Ba Yint Naung market. The sample came originally from Taunggyi Township, Southern Shan state.

Procedure

Ungerminated seeds of flint corn (250 g) were powdered by using a blender. The powder was suspended with 280 ml of 0.1 M acetate buffer pH 5. After the suspension had been stirred with magnetic stirrer for 5 hours at room temperature, it was filtered by using thin cotton cloth, and 160 ml of pale yellow extract (crude) was obtained. Solid ammonium sulphate (40 g) was added to the crude extract under stirring. The resulting precipitate was removed by ultra-centrifuging at 1,000 rpm for 30 min the supernatant 156 ml was obtained (first filtrate). Subsequently, solid ammonium sulphate (30 g) was slowly added to the supernatant. The resulting precipitate was collected by the ultracentrifuge at 1,200 rpm for 30 min and dried at room temperature and the enzyme powder (1.7 g) was obtained.

(b) Preliminary examination of α-glucosidase activity from flint corn seeds

Procedure

A test tube containing 1 ml of enzyme solution was labeled as tube A and another tube containing 1 ml of distilled water was labeled as tube B for blank solution.

4 mL each of 2% starch solution was added to the two test tubes A and B, then the contents were mixed well and kept for 30 min. After 30 min, 0.1 ml each of iodine solution was added to test tube A and B. Starch gives a deep blue colour with iodine in test tube B, whereas a test tube A containing solution mixture of enzyme and starch solution showed no blue colour with iodine solution.

(c) Determination of α-glucosidase inhibitory effect of crude extracts from Phan-khar fruits by α-glucosidase inhibition assay

The enzyme inhibition assay is based on the breakdown of substrate to produce a colour product, followed by measuring the absorbance over a period of time. In this experiment, the α-glucosidase inhibition activity of 95% ethanol and watery extracts selected plant samples was studied by inhibiting the breakdown of the sucrose to glucose.

Procedure

α-glucosidase inhibitory activity was determined by UV-visible spectrophotometric method. The control solution was prepared by mixing 1 ml of enzyme solution and 1 ml of substrate sucrose solution, and 0.5 ml of glucose oxidase solution in 1 ml of 6% DMSO solution. The background solution was prepared by mixing 1 ml of sucrose solution, 0.5 ml of glucose oxidase solution, 1 mL of buffer solution, and 1 ml of 6% DMSO solution. The test sample solution was prepared by mixing 1 mL of enzyme solution, 0.5 ml of glucose oxidase solution and, 1 ml of sucrose solution. This mixture was incubated in water-bath at 37°C for 5 min. And then 1 ml of sample solution was added and mixed. The reaction was carried out with incubation at 37°C for 30 min. After the incubation at 37°C for 30 min, the reaction was stopped by immersing all the test tubes in a boiling water bath for 10 min. All the tubes were allowed to cool at room temperature. Enzymatic activity was quantified by measuring the absorbance of the red pigment at 505 nm. Absorbance measurements of individual solution were used to calculate percent inhibition by the following equation.

$$\%\text{Inhibition} = \frac{[Ac-(A-Ab)]}{Ac} \times 100\%$$

Where, % Inhibition = %inhibition of test sample

A_c = absorbance of the control (sucrose + enzyme)

A_b = absorbance of the background (sucrose)

A = absorbance of test sample solution (sucrose + enzyme +sample)

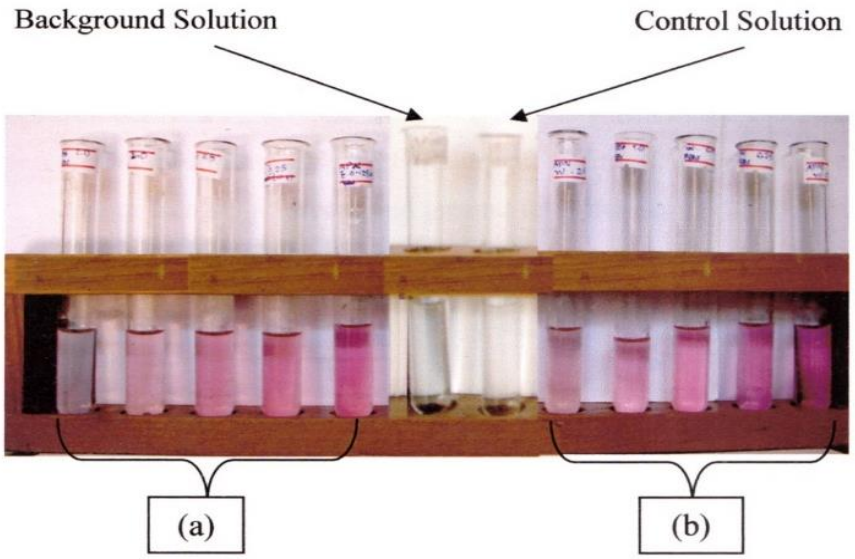
Then, IC_{50} (50% inhibitory concentration) value were also calculate by linear regressive excel program.

Average, $\bar{x}$ = $\frac{x1 + x2 + \cdots + xn}{n}$

Where, $\bar{x}$ = average % inhibition

$x_1 + x_2 + \ldots + x_n$ = % inhibition of test sample solution

n = number of times



(a) Ethanol extract : 2,1, 0.5, 0.25, 0.125 µg/ mL

(b) Watery extract :2,1, 0.5, 0.25, 0.125 µg/ mL

Figure 3. Prepared sample solutions for measurement of absorbance by UV-visible spectrophotometer (wavelength 505 nm)

Results and Discussion

Sample Collection and Preparation of Plants Materials

Collected and prepared dried powdered sample was stored in air tight containers so that the sample was free from getting molds and from other contaminations.

Preliminary Phytochemical Test

Preliminary phytochemical investigation was carried out to know the types of phyto-organic constituents present in selected Phan-khar fruits.

The phytochemical investigation of Phan-khar fruits indicated the presence of α-amino acids, glycosides, organic acid, phenolic compounds, reducing sugars, steroid, saponins, terpenoids, and tannins, whereas alkaloids, carbohydrates, cyanogenic glycosides, flavonoids, and starch, were not detected.

Amino acids are used as a precursor of a bioactive metabolite in combatting an infectious disease.

Glycosides a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. The glycosides, therapeutically have the ability to increase the force and power of the heart and the same time steady excess heart beats without strain to the organ.

Phenolic compounds are vital in defense responses, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities.

The presence of saponins supports the fact that Phan-khar fruits has cytotoxic effects. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning.

Steroids and terpenoids are bioactive compounds present in many plants. Antimicrobial and insecticidal properties of other terpenoids have led to their utilization as pesticides and fungicides in agricultural and horticulture. Table 1 showed preliminary phytochemical investigation results of Phan-khar fruits.

Crude Extracts from Phan- khar Fruits

After carrying out the preliminary phytochemical investigation tests, some crude extracts were firstly prepared. 0.3 % of petroleum ether (PE) extract, 31. 0 % of 95 % of ethanol (EtOH) extract, and 21.0 % of water extract were obtained. All these extracts were kept for further work. The resultant contents for various extracts are shown in Table 2.

Alpha- glucosidase Inhibitory Effect of Crude Extracts from Phan- khar Fruits

The α- glucosidase inhibitory effects of water and 95 % ethanol extract from Pan- khar fruits were determined by using α- glucosidase inhibition assay.

This method is based on the breakdown of sucrose substrate by α- glucosidase to produce *β*- D- glucose, followed by measuring the absorbance was carried out at wavelength 505 nm using UV- visible spectrophotometer.

In this experiment, five different concentration (0.125 µg/ mL, 0.25 µg/ mL, 0.5 µg/ mL, 1.0 µg/ mL, and 2.0 µg/ mL) for each crude extract were prepared in aqueous solution. Volglibose was used as a standard drug and sucrose solution was employed as substrate. Volglibose may have additional utility in the management of type II diabetes. Despite the clinical use of Voglibose, the mechanism of its action is not fully understood.

The absorbance and percent inhibitory values of crude extract were measure at different concentrations. Increase in inhibitory effect of crude extracts usually expresses in term of % inhibition.

The inhibitory effect of the sample on α- glucosidase is usually determined by IC_{50} (50 % of inhibition concentration) values in µg/ mL were then calculated from % inhibition by linear regressive excel program. The results are summarized in Table 3, Figure 4, and Figure 5.

From these experimental results, it was observed that as the concentration of the test samples were increased, the absorbance values were decreased, i.e., increase in α- glucosidase inhibitory effect.

The 50 % of inhibition concentration (IC_{50}) of Phan- khar water extract and Phan- khar 95 % ethanol extract were found to be 0.52 µg/ mL and 0,43 µg/ mL. On the basic of these IC_{50} values, the inhibitory effects of Phan- khar water extract and its ethanol extract on α- glucosidase inhibitory effect were found to be different to each other. But it was observed that the water and 95 % ethanol extract of plant samples showed lower potency than standard drug Voglibose (IC_{50} = 0.32 µg/ mL) in α- glucosidase inhibitory effect. The IC_{50} values of crude extracts and Voglibose were also describe in Figure 6.

The order of α- glucosidase inhibitory effect of crude extracts were observed to be as Phan- khar 95 % ethanol extract (IC_{50} = 0.52 µg/ mL) > Phan- khar water extract (IC_{50} = 0.52 µg/ mL).

Table 1. Preliminary Phytochemical Investigation Results of Phan-Khar Fruit Sample (*Terminalia chebula* Retz)

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloids	1% HCl	(i) Dragendeoff reagent	no ppt	-
			(ii) Sodium picrate	no ppt	-
			(iii) Wagner reagent	no ppt	-
			(iv) Mayer reagent	no ppt	-
2.	Alpha-amino acid	H_2O	Ninhydrin reagent	Violet spot	+
3.	Carbohydrate	H_2O	10% alpha-naphthal and H_2SO_4	No colour change	+
4.	Cyanogenic glycosides	H_2O	Conc: H_2SO_4 & Sodium picrate paper	No brick- red colour	-
4.	Flavonoids	EtOH	Mg ribbon and con: HCl	Pink colour	-
5.	Glycosides	H_2O	10% lead acetate	White ppt	+
6.	Phenolic compound	H_2O	10% $FeCl_3$	Blue-black colour	+
7.	Steroids/Terpenoids	PE	Acetic anhydride & conc: H_2SO_4	Blue	+
8.	Starch	H_2O	1% I_2	Blue colour	-
9.	Tennin	H_2O	Gelatin 10% $FeCl_3$	Deep blue	+
10	Reducing sugar	H_2O	Benedit's solution	Green	+
11.	Saponin	H_2O	H_2O	Frothing	+
12.	Organic acid	H_2O	Bromocresol green	Green colour	+

(+) present, (-) absent

Table 2. Yield Percent of Crude Extracts from the Phan- khar Fruit

Sample	Yield percent of crude extracts (% W/ W)		
	PE (60- 80) extract	95 % EtOH extract	Watery extract
Phan- khar fruits	0.2	31.0	21.0

Table 3. Percent Inhibition of Various Concentrations and IC_{50} Values of Two Crude Extracts from Phan- khar Fruits and Standard Voglibose on Alpha- glucosidase Inhibitory Effect

Test samples	Percent inhibition					IC_{50} (µg/ mL)
	0.125	0.25	0.5	1. 0	2. 0	
Watery extract	30.59	38. 61	49.58	58.65	62. 03	0.52
95% EtOH extract	29.74	43. 37	52. 53	55. 48	61. 18	0.43
Standard Voglibose	31. 06	48. 22	55. 41	58. 33	64. 39	0. 32

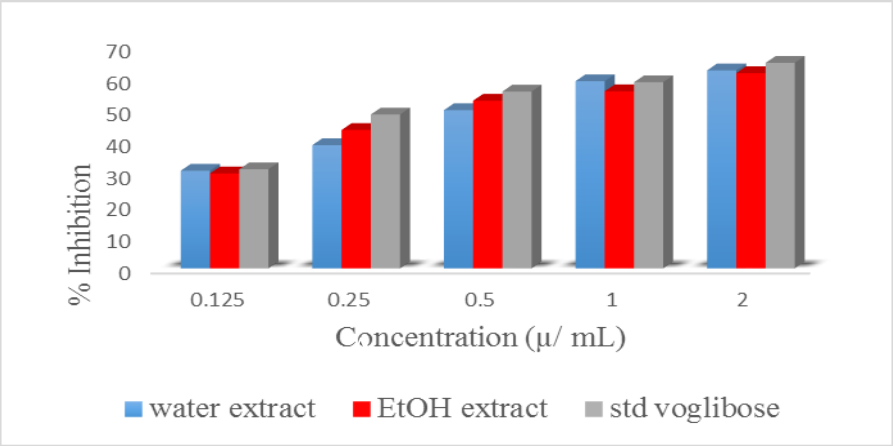


Figure 4. Diagram for % inhibition Vs concentration of water and 95 % ethanol extracts from Phan- khar fruits and standard voglibose on alpha-glucosidase inhibitory effect

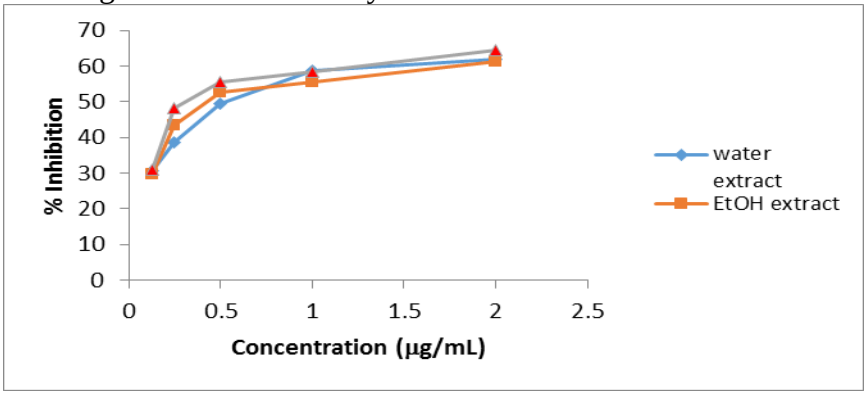


Figure 5. A plot of % Inhibition Vs concentration of water and 95 % ethanol extracts from Phan- khar fruits and standard voglibose on alpha-glucosidase inhibitory effect

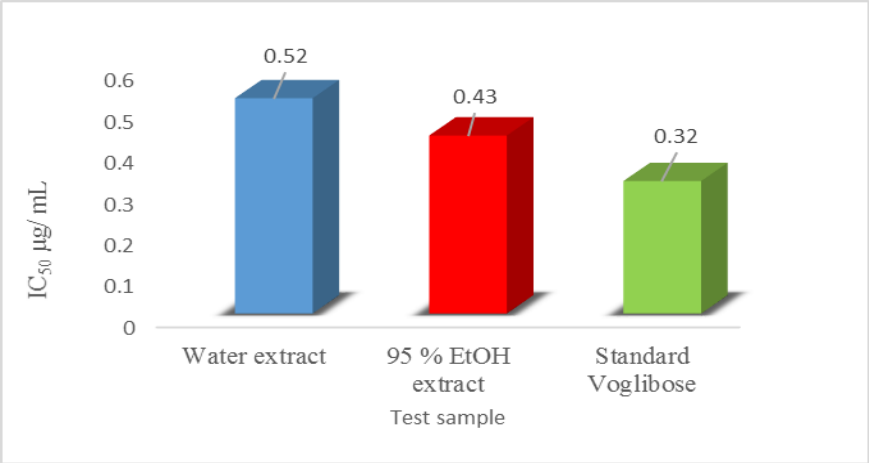


Figure 6. IC_{50} values of water and 95 % ethanol extracts from Phan- khar fruits compared with standard voglibose on alpha- glucosidase inhibitory effect

Conclusion

In the present research work, the following inference may be deduced from the overall assessment.

The phytochemical investigation of Phan-khar fruits indicated the presence of α-amino acids, glycosides, phenolic compounds, reducing sugars, saponins, steroid, terpenoids, tannins, and organic acids whereas, alkaloids, carbohydrates, cyanogenic glycosides, flavonoids, and starch were not detected.

The alpha- glucosidase inhibitory effect of watery extract and 95 % ethanol extract were determined. alpha- glucosidase inhibitory assay method revealed that IC_{50} of water extract and 95 % ethanol extracts were 0.52 µg/ mL and 0.43 µg/ mL, respectively. The test results revealed that ethanol extract was more effective alpha-glucosidase inhibitory than watery extract.

Standard Voglibose, synthetic medicine was used as a standard reference which showed IC_{50} of 0.32 µg/ mL. The test samples of *Terminalia chebula* Retz. (Phan- khar) fruits showed mild activity when compared to the synthetic medicine.

However, it is hoped to be fact that the *Terminalia chebula* Retz. (Phan- khar) fruits can be used as a ∞- glucosidase inhibitor for treatment of diabetes as a natural product.

Phan- khar fruits, that are grown in Myanmar, in an abundant and inexpensive can be used as anti- diabetes readily available daily for alpha- glucosidase inhibitory.

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