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Research Article

α-GLUCOSIDASE INHIBITION ACTIVITY OF STANDARDIZED EXTRACT OF *ALOE VERA* COLLECTED FROM YAYO BIOSPHERE RESERVE FOREST, ETHIOPIA

Desalegn Chilo [1], Javed Ahamad [1]*, Solomon Yeshanew [1], Rajakumar P [1], Naila Hassan [2], Showkat R Mir [2]
[1]Department of Pharmacy, Faculty of Public Health & Medical Sciences, Mettu University, Mettu, Ethiopia
[2]Department of Pharmacognosy, Faculty of Pharmacy, Hamdard University, PO Hamdard Nagar, New Delhi, India
*Corresponding Author Email: jas.hamdard@gmail.com

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ABSTRACT

In recent years, there has been an increased interest in the development of alternative medicines for maintaining glucose homeostasis in diabetes, specifically by screening plant extracts as well as their isolated compounds for their ability to delay or prevent glucose metabolism and absorption. The objective of present study was to evaluate the *in-vitro* α-glucosidase inhibitory activity of standardized extract of *Aloe vera*. In this study, we standardized ethanolic extracts of *Aloe vera* by using thin layer chromatography (TLC) and UV-Visible spectroscopy and then standardized extract was selected for *in-vitro* α-glucosidase inhibitory assay. Standardized ethanolic extract of *Aloe vera* showed concentration dependent inhibition of α-glucosidase varying from 13.96±0.67 to 76.10%±0.84 % for 100 to 1000 µg/mL, respectively. The IC_{50} values for *Aloe vera* extract and acarbose were found as 530±7.18 and 265±5.31 µg/ mL, respectively. The present study indicated the potential of *Aloe vera* to prevent postprandial hyperglycemia by inhibition of α-glucosidase enzyme.

Keywords: *Aloe vera*, Liliaceae, α-glucosidase, standardization, PPHG.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the healthcare system. It is associated with several other metabolic abnormalities such as obesity, hypertension, and dyslipidaemia, which contributes to the very high rate of cardiovascular morbidity and mortality[1-3]. Insulin is the principle hormone that regulates uptake of glucose into most of cells from the blood. Deficiency of insulin or the insensitivity of its receptors plays a central role in diabetes mellitus[4]. The number of people in the world with diabetes has increased dramatically over the recent years. The glucose homeostasis is one of the pivotal therapeutic modality in the management diabetes. Normally it is maintained by a fine balancing act of insulin and glucagon in the body. Hydrolytic enzyme α-glucosidase is involved in the metabolism of polysaccharides to oligosaccharides and ultimately to glucose that is amenable to intestinal absorption. Inhibition of α-glucosidase enzyme lowers the amount of glucose entering the systemic circulation, while most of the carbohydrate from the diet leaves GIT undigested. Therefore, attaining glucose homeostasis through the inhibition of carbohydrate metabolizing enzymes is an interesting strategy as it excludes the involvement of insulin (pancreas). It attains glucose metabolism and regulation beyond insulin and glucagon. Acarbose, voglibose and miglitol are the inhibitors of carbohydrate metabolizing enzymes used clinically to control postprandial hyperglycemia (PPHG) in diabetics[5,6]. Traditional medicines are known to have preventive and therapeutic effects in diabetes, but their active components have not yet been characterized, except in a few cases. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated[7]. Traditional antidiabetic formulations, consisting of herbal decoctions, are required for a long term use besides being considered ineffective in chronic conditions. The main reason being that these remain sub-therapeutic as these contain many components with a less or even without any effect. Moreover, a multi-component formulation is often difficult to be standardized, but inclusion of only active extracts/fractions in the formulation makes it easier to be standardized with respect to its bio-active components and activity[8].

The genus *Aloe* (Liliaceae) contains over 180 species of flowering succulent plants, among which the most widely known species is *Aloe vera* (L.) Burm. f. or true aloe, which is one of the most important pharmaceutical herbs[9,10]. *Aloe vera* is a traditional remedy for diabetes mellitus (DM) in many parts of the world, including Latin America[11] and the Arabian Peninsula[12]. Aloes contain C-glycosides and resins. Aloe is the major source of the anthraquinone glycosides like aloin (figure 1) which is mixture of glucosides among which barbaloin is the chief constituent. Barbaloin is a C-glycoside - a 10-glucopyranosyl derivative of aloe emodin-Anthrone. It is also contain isobarbaloin, *β*-barbaloin, aloe emodin and resin (principle compound is Aloesin). Chrysophanic acid, chrysamminic acid, aloetic acid etc. are also other constituents of aloe. The substance inside the leaf called gel consists of 99% water with long chain polysaccharide, of acetylated glucomannan kind, and other carbohydrates[13]. It was claimed that the polysaccharides in *Aloe vera* gel had therapeutic properties such as anti-inflammatory, wound healing, promotion of radiation damage repair, antidiabetic and anti-neoplastic activities[14]. The dried sap, of the

Aloe plant had been used for diabetes in the Arabian Peninsula[15]. Its ability to lower the blood glucose was studied in five patients with non insulin dependent diabetes and in Swiss albino mice made diabetic using alloxan[16]. The hypoglycemic activity of Aloe species was first demonstrated by Agrawal[17]. Since then, the antidiabetic effects of Aloe preparations have been demonstrated in diabetic patients[18-21].

MATERIALS AND METHODS

Plant material

Fresh leaves of *Aloe vera* (sample no. 2) were collected in March 2017 from Yayo Biosphere Reserve Forest, Ethiopia and other samples of *Aloe vera* (1, 3, 4 and 5) were collected from local crude drug market; and all samples authenticated in Department of Pharmacy, Faculty of Public Health and Medical Sciences, Mettu University, Mettu, Ethiopia. Voucher specimen of the each sample was deposited in the Department of Pharmacy, Faculty of Public Health and Medical Sciences, Mettu University, Mettu, Ethiopia.

Chemicals and reagents

α-Amylase, α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (PNPG) and 3,5-dinitrosalicylic acid (DNS) were purchased from SRL, Bangalore, India. Precoated silica gel 60 F_{254} TLC plates were purchased from Merck, India. Acarbose was obtained as gift sample from Medley Pharmaceutical Ltd. Jammu, India. All other solvents and chemicals were of analytical grade.

Standardization of *Aloe vera* extracts

TLC Identity Test for *Aloe vera* samples

Preparation of test solutions

Weight accurately 5 g crude drug powder separately and refluxed for 2 hr with ethanol (100 mL). The extract was filtered through Whatman filter paper and the filtrate was concentrated up to 10 mL on water bath. These sample solutions were further used for UV-visible spectroscopic studies and α-glucosidase inhibition assay.

Solvent system

Mobile phase: ethyl acetate: methanol: water (80: 15: 5 *v/v*).

TLC Procedure

Apply 4 µL of the test solutions of different samples separately on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in the solvent system in a twin trough chamber to a distance of 8 cm.

Visualization

Observed the plate after spraying with 10% ethanolic KOH reagent followed by heating the plate at 105º for 5 to 10 min. Record the R_f value and colour of the resolved bands.

UV-Visible spectroscopic test for *Aloe vera* samples

Weight accurately 5 g crude drug powder and refluxed for 2 hr with ethanol (100 mL). The extract was filtered through Whatman filter paper and the filtrate was concentrated up to 10 mL on water bath. Further test solutions are diluted with ethanol till the samples showing optical density (OD) less than 1. For each test samples UV spectra were recorded from 250 to 350 nm using UV-visible spectrometer (Shimadzu, Japan).

In-vitro α-glucosidase inhibition assay

The enzyme inhibition assays were carried out with standardized ethanolic extract of *Aloe vera* and acarbose at doses ranging from 100 to 1000 µg/mL as per the method given by Ahamad *et al.,*[8], with slight modifications. Briefly, a volume of 60 µL of sample solutions in DMSO of *Aloe vera* extract or acarbose and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) was incubated in 96 well plate at 37 ºC for 20 min. After pre-incubation, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 ºC for another 20 min. Then the reaction was stopped by adding 160 µL of 0.2 M Na_2CO_3 into each well, and absorbance (A) recorded at 405 nm by micro-plate reader and compared to a control which had 60 µL of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as percent inhibition and was calculated as follows:

$$\%\ Inhibition = \frac{\text{A control} - (\text{A } test - A\ background)}{\text{A control}} \times 100$$

where $A_{control}$, A_{test}, $A_{background}$ are defined as the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, respectively.

The concentration of inhibitors required for inhibiting 50% of enzyme activity under assay conditions was presented as IC_{50} value.

RESULT AND DISCUSSION

TLC results of *Aloe vera* samples

Precoated TLC plates developed by using mobile phase ethyl acetate: methanol: water (80: 15: 5 *v/v*) up to 8 cm and it gave well resolved bands after derivatization with 10% ethanolic KOH reagent followed by heating the plate at 105º for 5 to 10 min (figure 2). A prominent band at R_f 0.45 corresponding to Aloin[22] is visible in test sample no. 2 (collected from Yayo Biosphere Reserve Forest, Ethiopia). Test sample no. 1 and 3 also showing bands for aloin (R_f 0.45) but with less density. Sample no. 4 shows bands for aloin (R_f 0.45) but the visibility is very less. *Aloe vera* sample no. 5 does not show bands for aloin the major constituents of *Aloe vera* (Table 1, Figure 2). On the basis of TLC results we can conclude that *Aloe vera* sample no. 2 collected from Yayo Biosphere Reserve Forest, Ethiopia is authentic sample containing major phytochemicals of Aloe that is aloin.

UV spectroscopic results of *Aloe vera* samples

The ethanolic extracts of *Aloe vera* (sample no. 1, 2, 3, 4 and 5) were scanned by using UV-visible spectrometer from 250 to 350 nm using. The UV results were presented in Figure 3. The reference value of λ_{max} for ethanolic extract of *Aloe vera* is given Homoeopathic Pharmacopoeia of India (HPI), at λ_{max} 278, 310 nm. On the basis of UV scan the *Aloe vera* sample no. 1 showing λ_{max} at 283 nm and 270 nm, sample no. 2 showing λ_{max} at 279 nm, sample no. 3 showing λ_{max} at 280.40 nm, sample no. 4 showing λ_{max} at 281.70 nm and sample no. 5 showing λ_{max} at 269.50 nm. So our UV results of *Aloe vera* sample no. 2 showing λ_{max} at 279 nm which is comparable with reference λ_{max} of HPI.

Table 1. TLC Details of Test Solution of *Aloe vera* after derivatization with 10% ethanolic KOH reagent

R_f values of *Aloe vera* samples					Colour of the band
1	2	3	4	5	
0.45	0.45	0.45	0.45	-	Bluish yellow (Aloin)
0.51	0.51	0.51	0.51	-	Light blue
0.86	0.6	0.86	0.86	0.86	Blue

Table 2. *In-vitro* α-glucosidase inhibitory activity of ethanolic extract of *Aloe vera* (sample no. 2)

Concentration (µg/mL)	Percent inhibition	
	Acarbose	*Aloe vera*
100	20.37 ± 0.61	13.96 ± 0.67
200	44.38 ± 0.86	24.42 ± 1.96
400	61.28 ± 1.38	48.33 ± 0.98
600	73.10 ± 1.51	65.21 ± 1.37
800	81.43 ± 1.88	71.61 ± 0.41
1000	86.25 ± 0.84	76.10 ± 0.84
IC_{50} value	265±5.31 (µg/mL)	530±7.18 (µg/mL)

Data expressed as Mean ± SD, n = 3;
IC_{50} = concentration for 50% inhibition;

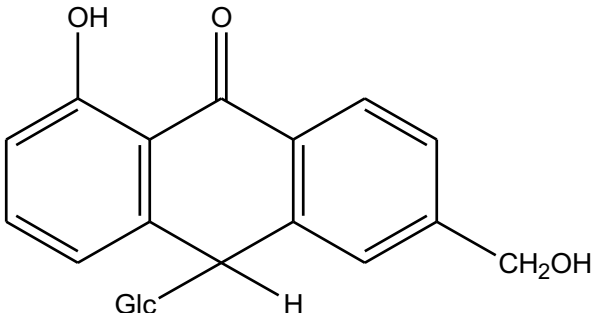

Figure 1. Chemical structure of Aloin

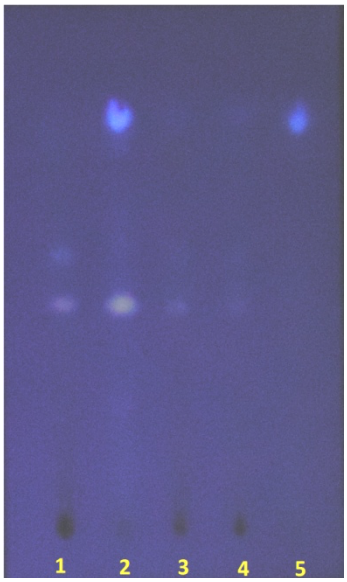

Figure 2. TLC profile of test solution of *Aloe vera* after derivatization with 10% ethanolic KOH reagent

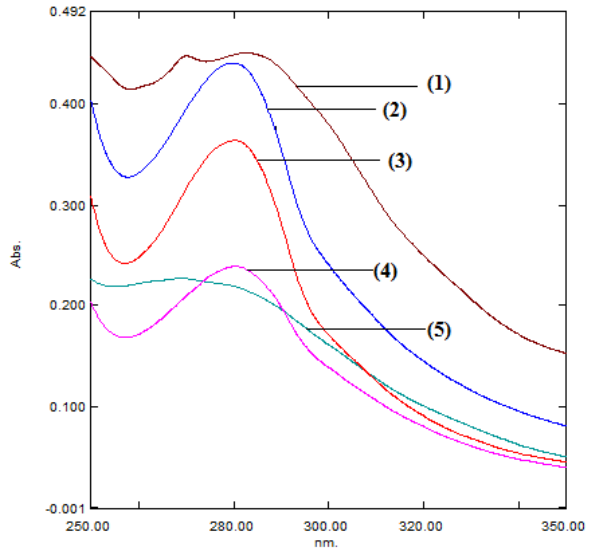

Figure 3. UV-Visible spectra of ethanolic extracts of *Aloe vera* samples

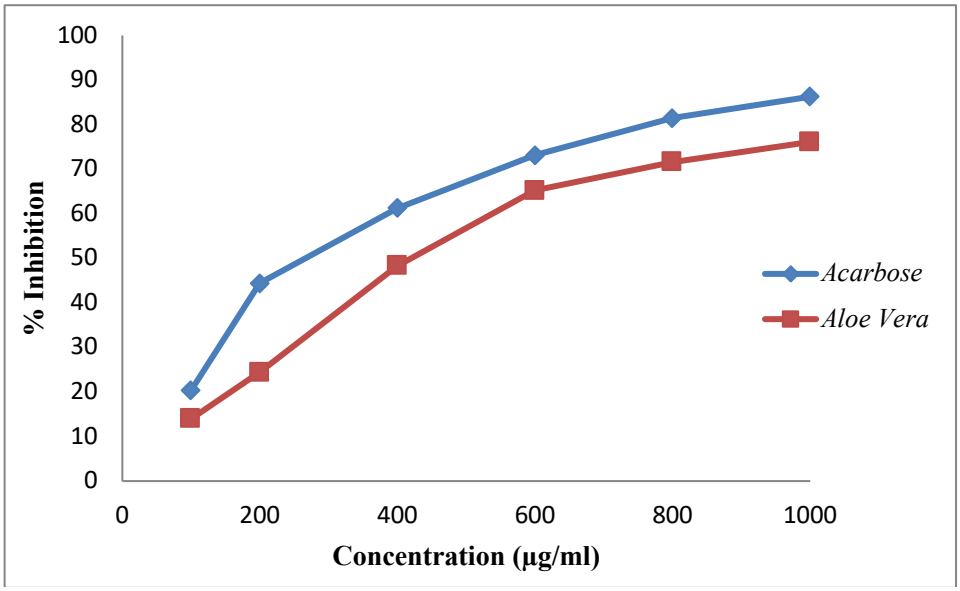

Figure 4. ***In-vitro*** **α-glucosidase inhibition by Acarbose and ethanolic extract of** ***Aloe vera*** **(sample no. 2)**

α-Glucosidase inhibition activity of *Aloe vera*

The results of α-glucosidase inhibitory study are shown in Figure 4. Standardized ethanolic extract of *Aloe vera* (sample no. 2) showed concentration dependent inhibition of α-glucosidase varying from 13.96±0.67 to 76.10%±0.84 % for 100 to 1000 µg/mL, respectively. Acarbose also showed a concentration dependent response that varied from 20.37±0.61 to 86.25±0.84% for 100 to 1000 µg/mL, respectively. The IC_{50} values for *Aloe vera* extract and acarbose were found as 530±7.18 and 265±5.31 µg/ mL, respectively.

CONCLUSION

The present study we standardized *Aloe vera* extracts by using TLC and UV-visible spectroscopy and we found the *Aloe vera* sample no. 2 showing TLC spots for its major chemical constituents that is aloin and also it showing UV spectra comparable with reference value. Hence, we chose *Aloe vera* sample no. for α-glucosidase inhibition assay. In our study standardized ethanolic extract of *Aloe vera* (sample no. 2) showed strong α-glucosidase inhibitory activity.

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