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Antidiabetic Activities of Endangered Plants of Western Ghats

Dr. Sumanagala N[1], Dr. Jayaramu [2], P. Suman[3], Sukeerthi N [4]

Assistant Professor, Department of Microbiology, BMS College for Women, Bengaluru, Karnataka[1]

Research Supervisor, Asso. Professor, (Retd), Department of Studies and Research in Environmental Science, Tumkur University, Tumakuru, Karnataka[2]

Assistant Professor, Department of Microbiology, Government Science College (Autonomous), Bengaluru, Karnataka[3]

UG Student, St. Joseph's College (Autonomous), Bengaluru, Karnataka[4]

Abstract: Diabetes mellitus has become one of the biggest health problems around the globe. Thus, in recent times, the demand for affordable and efficient antidiabetic medications has increased considerably. The present study aims to evaluate the antidiabetic activities of methanolic leaf extracts of endangered plants of western ghats. Enzymes in the intestine namely, alpha-glucosidase plays an important role in the digestion of carbohydrates and in controlling the glucose level in blood. α-Glucosidase inhibition bio-assay was conducted by following the standard protocol using Glucose Oxidase method with Acarbose as the standard and the absorbance was measured at 510 nm. In α-Glucosidase inhibition bio-assay, the plant samples Curcuma zedoaria, Syzygium travancorium stem and Plectranthus vettiveroides leaves showed inhibition activity against α-glucosidase with increased extract concentration. Among the three samples, S. travancorium stem extract was proven most antidiabetic hence making it a possible source in the preparation of antidiabetic drugs. The antidiabetic activity is directly proportional to the concentration of the sample extracts since the obtained inhibition values are increased with the increased concentration. S. travancorium, with higher inhibition activity, was observed to be the best source for antidiabetic activity among all the tested samples.

Keywords: Diabetes mellitus, α-Glucosidase, antidiabetic, inhibition activity, Western ghats, endangered plants

1. INTRODUCTION

Around 2,65,000 seed plant species are present on earth and less than 50% of these have been examined for chemical configuration and medicinal value. The conservation of threatened medicinal plants species of the wild is indispensable [1]. Most of the known plants species with medicinal properties are categorized under RET (Rare Endangered Threatened) list. As in any other tropical region around the world, in India too, the forests are highly fragmented and non-contiguous because of human intervention and intense developmental activities over a last few decade [2]. Wiping natural vegetation for agricultural purposes and introducing alien species, soil erosion and salinization are some of the main factors for the extinction of plant species but the major significant threat of climate change is adding on to the same [3]. About 1052 plant species are Red-listed in India many of which are in the list of medicinal plant species [4].

1.1 Reason for Endangering of Plants

Most of the economically important plants are usually assumed to be semi-public products in India because of which the harvesting is un-regulated from the forest [2]. Among the species of medicinal plant species globally, 58 species of the Western Ghats region alone are extremely endangered because of excess of harvesting, according to the analysis of the Rare Endangered Threatened [RET] status of medicinal plants [2]. The pharmacological efficiency of the medicinal plant extracts can be studied by separating out the active components [5]. Plants are found to be the richest source of medicinal drugs which play an essential role in the scope of traditional medicines, nutraceuticals, modern medicines and synthetic drugs [6].

1.2 Anti-diabetic Properties

Diabetes mellitus is a well-known endocrine ailment that has brought about a very high death rate worldwide owing to the many micro and macro vascular complications it can induce [7]. WHO estimates that by 2030 diabetes could become the seventh most common cause of death. The disease is rapidly increasing world over and affecting people all over the Globe. Deficiency in the production of insulin causes the glucose level in blood to raise abnormally in diabetes patients [8]. Presently the conventional medication for people suffering from this condition is insulin and other antidiabetic agents that can be ingested orally, for instance glinides, sulfonylureas and biguanides. But these drugs can cause a variety of side

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effects and thus, scientists are constantly researching natural compounds that could be powerful and reliable hypoglycemic agents [9].

Most of these plants contain phytoconstituents like glycosides, carotenoids, alkaloids, flavonoids, terpenoids, etc., which are often proved to have anti-diabetic effects [10]. The ethnobotanical information guide suggests that approximately 800 species might be capable of showing anti-diabetic effects [8].

The number of plant species having hypoglycemic activity exceeds 400 [11].

Diabetes mellitus is a well-known endocrine ailment that has brought about a very high death rate worldwide owing to the many micro and macro vascular complications it can induce [7].

Many therapeutic agents are available in medicine to treat diabetes, but they are expensive and toxic [13]. Synthetic drugs can cause side effects such as insulinoma, kidney dysfunction, gastrointestinal problems and hypoglycemia .Thus, scientists are constantly researching natural compounds that could be powerful and reliable antidiabetic agents with no toxicity. [9].

India provides the best quality and quantity of medicinal plants and stands second in ranking in terms of export. It is considered as one of the 12 mega biodiversity hotspots of the world with 16 agro-climatic zones and has wide range of about 45,000 plants out of which 7000 plant species are recognized as medicinal plants [14].

The enzyme in the intestine, alpha-glucosidase play an important role in the digestion of carbohydrates and are proven to control the level of glucose in blood. Inhibiting this enzymes is thus an effective method in the management of post prandial blood glucose level in a type 2 diabetic patient. [15].

Isolation and identification of bioactive phytochemicals from the plants play an important role in improving insights into anti-diabetic functional food [18] and drug development [19].

2. MATERIALS AND METHODS

2.1 Preparation of Enzyme

The rat intestine was taken and cooled with 80mM phosphate buffer (pH 7.0) at ice cold temperature. A glass rod was used to scrape off the mucosa off of the intestine. It was then kept in the homogenizer with four parts volume-by-weight of the buffer. Crushed ice was added to the tube while homogenization is being done. Centrifugation is carried out at around 3000 rpm for ten minutes to discard larger cell debris. The supernatant was kept at -20°C. Reference inhibitor: 50mg of acarbose is added to 50ml of phosphate buffer and appropriately diluted using the same buffer (pH 7.0) in order to obtain 5µg/ml as the final concentration.

2.2 α-Glucosidase inhibition bio-assay

Determination of the effect of plant samples on α-glucosidase activity was carried out by the process as interpreted by Kim et al., 2005 [23].

Preparation of Enzyme: 250µl of the buffer solution was mixed with 50µl of the above prepared enzyme and this product was incubated for 30 minutes at 37°C. 500µl sucrose solution was then added to this and the final mixture was again incubated twenty minutes at 37°C. Then it was heated on a boiling water bath for around two minutes to arrest the reaction. The mixture was then cooled and the concentration of glucose was measured by the Glucose Oxidase process. Glucose estimation: 100µl of the sample and 500µl of the glucose reagent from the kit are mixed, incubation was carried out at lab temperature for around ten minutes. The absorbance was measured at 510nm.

Percentage inhibition of α-glucosidase is calculated using the below formula:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad (1)$$

3. RESULTS

3.1 α-Glucosidase Inhibition Bio-Assay

α-Glucosidase inhibition bio-assay was conducted by following the standard protocol using Glucose Oxidase method with Acarbose as standard and the absorbance was measured at 510 nm. The results are interpreted in Table 1 and Figures 1 and 2. The anti-diabetic activity is directly proportional to the concentration of the sample extracts because, the obtained inhibition values are increased with the increased concentration. So, the anti-diabetic activity is dose dependent factor. Again, the IC50 value from the inhibition percentage and it is the concentration required to inhibit the 50 percent α-glucosidase enzyme. The lesser the IC50 value the efficiency of the extract will be higher in the inhibition activity. In α-Glucosidase inhibition bio-assay the three plant samples C. zedoaria, S. travancoricum stem and P. vettiveroides leaves showed increased inhibition activity against α- glucosidase with increased extract concentration.

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Among the three samples S. travancoricum stem extract showed IC50 value of 150.1 µg/ml with standard acarbose with IC50 value of 0.1867 µg/ml and for other two samples IC50 value was not calculated due to lesser inhibition activity. At the maximum concentration of 200 µg/ml S. travancoricum stem has showed the anti-diabetic activity of 60.34 µg/ml. Hence the S. travancoricum sample will be the best source for anti-diabetic activity among all the tested samples with higher inhibition activity. Therefore, the sample will be very good source in the preparation of antidiabetic drugs.

Table 1: α-Glucosidase inhibition bio-assay of Acarbose (standard), C. zedoaria stem, S. travancoricum stem and P. vettiveroides leaf.

Compound name	Conc. µg/ml	OD at 590nm	% inhibition	IC_{50} µg/ml
Control	0	0.886	0	
Acarbose	0.0781	0.964	30.66	0.1867
	0.1562	0.742	46.59	
	0.3125	0.637	54.2	
	0.625	0.392	71.83	
	1.25	0.337	75.73	
	2.5	0.248	82.17	
C. zedoaria stem	6.25	0.816	7.86	IC 50 was not calculated due to lesser percentage inhibition
	12.5	0.781	11.9	
	25	0.750	15.32	
	50	0.707	20.17	
	100	0.682	23.01	
	200	0.642	27.49	
S. travancoricum stem	6.25	0.794	10.39	150.1
	12.5	0.770	13.09	
	25	0.709	19.98	
	50	0.606	31.56	
	100	0.513	42.13	
	200	0.351	60.34	
P. vettiveroides leaves	6.25	0.805	9.09	IC 50 was not calculated due to lesser percentage inhibition
	12.5	0.785	11.42	
	25	0.763	13.85	
	50	0.736	16.9	
	100	0.704	20.54	
	200	0.539	39.13	

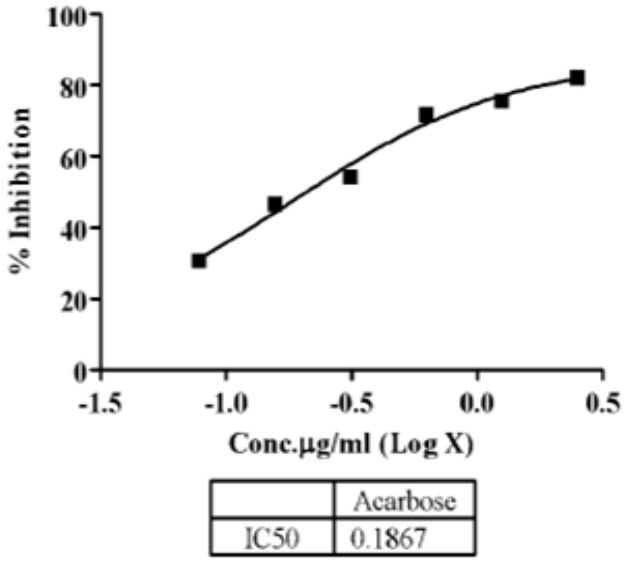

Fig 1. α- Glucosidase inhibition bio-assay determination of IC50 value for Acarbose (standard)

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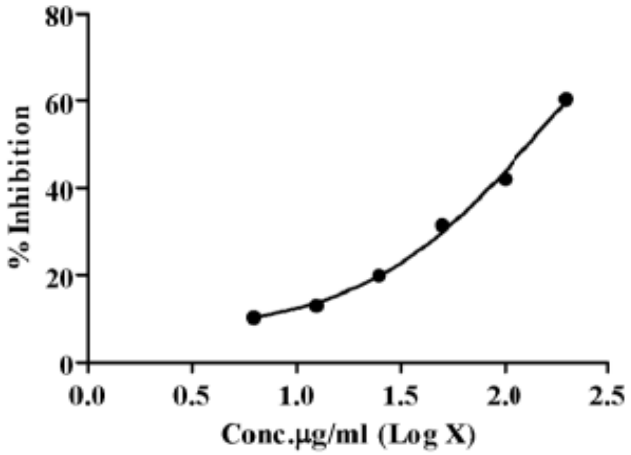

IC50	150.1

Fig 2. α- Glucosidase inhibition bio-assay determination of IC50 value for S. travancoricum sample

CONCLUSION

Various studies have suggested the role of plant extracts as α-glucosidase inhibitors clearly indicating the potential of these extracts to manage hyperglycemia.

In this study, α-Glucosidase inhibition bio assay of the three plant samples C. zedoaria, S. travancoricum stem and P. vettiveroides leaves showed inhibition activity against α- glucosidase with increased extract concentration. Among the three samples S. travancoricum stem extract showed IC50 value of 150.1 µg/ml in α- Glucosidase inhibition bio assay.

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