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A Study on Evaluation of *In-Vitro* Antidiabetic Activities of Endangered Plant Species *Syzygium Travancoricum* of the Western Ghats

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KEYWORDS

S. travancoricum, stem extract, Antidiabetic, Alpha-glucosidase, Alpha-amylase

ABSTRACT:
Diabetes mellitus is a complex, irreversible metabolic illness that affects insulin secretion, function, or both, and raises blood sugar levels. Even now, acarbose and voglibose, either alone or in combination with insulin are used as inhibitors of the enzymes that break down carbohydrates. Nevertheless, these substances have been linked to adverse side effects. Therefore, this study aimed for evaluation of in-vitro antidiabetic activities of some endangered plant species collected from the western ghats. Results revealed that the stem extract of Syzygium travancoricum at a concentration range of 0.0781µg/mL, 0.1562µg/mL, 0.3125µg/mL, 0.625µg/mL, 1.250µg/mL, and 2.500µg/mL showed inhibition effect of 30.66%, 46.59%, 54.20%, 71.83%, 75.73%, and 82.17% respectively in alpha-glucosidase inhibition activity. The IC50 value of stem extract of S. travancoricum in alpha-glucosidase inhibition activity was found to be 150.10µg/mL in comparison with the standard antidiabetic drug acarbose with an IC50 value of 0.19µg/mL. Similarly, stem extract of S. travancoricum at a concentration range of 0.0781µg/mL, 0.1562µg/mL, 0.3125µg/mL, 0.625µg/mL, 1.250µg/mL, and 2.500µg/mL showed inhibition effect of 30.48%, 46.49%, 54.06%, 71.72%, 75.72%, and 82.12% respectively in alpha-glucosidase inhibition activity. The IC50 value of stem extract of S. travancoricum in alpha-amylase inhibition activity was found to be 48.38µg/mL in comparison with the standard antidiabetic drug acarbose with an IC50 value of 0.19µg/mL. In conclusion, stem extract of S. travancoricum exhibited considerable anti-diabetic properties, and hence this study supports the employment of stem extract of S. travancoricum in the development of antidiabetic medicinal drugs.

INTRODUCTION

Among the serious metabolic disorders, diabetes mellitus is a most critical disorder throughout the world with India listed in the top three countries. It is life threatening and responsible for many complications (such as retinopathy, neuropathy, and angiopathy) affecting various organs in the body, especially the eyes, followed by dysfunction and failure of various functional organs.[1] Genetic and environmental factors contribute significantly to the development of diabetes.[2] During the development of diabetes, the cells of the body cannot metabolize sugar properly due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (a peptide hormone that regulates blood glucose). The inability of insulin to metabolize sugar occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. This triggers the

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body to break down its own fat, protein, and glycogen to produce sugar, leading to the presence of high sugar levels in the blood with excess by-products called ketones being produced by the liver.[3,4]

The universal prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. Moreover, the prevalence of diabetes has also been found to steadily increase for the past 3 decades and has risen faster in low- and middle-income countries compared to high-income countries. The increase in the prevalence of diabetes is parallel with an increase in associated risk factors such as being overweight or obese. If not properly treated or controlled, diabetes may cause blindness, kidney failure, lower limb amputation, and other long-term consequences that impact significantly on the quality of life.[5] Interestingly, the WHO also projects that diabetes will be the seventh leading cause of death in 2030.[6]

The naturopathic treatment for diseases has been explored extensively since ancient times and gaining momentum in the present scenario.[7] Indian flora accounts for about 45,000 plant species out of which several thousands have pharmacological significance.[8] Plants are a great concern for drug discovery exploration and a major source of our modern medicine. About 25% of modern medicines are derived from a plant source and merely 5-15% of plants have been investigated for their medicinal use. Nowadays, natural plants, herbal medicines, phytomedicines and functional foods are extensively studied by scientists all over the world which resulted with the lucrative therapeutic potentials such as antidiabetic, anticancer, immunomodulating, antiobesity and lipid lowering, anti-inflammatory and anti-bacterial activities.[7]

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. 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 status of medicinal plants.[9] The pharmacological efficiency of the medicinal plant extracts can be studied by separating out the active components.[10] 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.[11]

With this scenario, in the current study we aimed for evaluation of *in-vitro* antidiabetic activities of some endangered plant species collected from the western ghats.

MATERIALS AND METHODS

Collection of Plant Species

The plant species *viz. Syzygium travancoricum* (Figure 1) selected in the present study were collected from the Foundation for Revitalisation of Local Health Traditions (FRLHT), Yelahanka, Bengaluru. The plants were classified and authenticated for taxonomic identity. The plants and their parts were collected in plastic bags and transported to the laboratory. Roots, stem and leaves are shade dried, powdered and stored in refrigerator until the time of analysis.

Figure 1. Showing plant species collected from FRLHT, Yelahanka, Bengaluru

Extraction

The collected samples were separated into leaves, stem and roots. All the parts were cleaned and shade dried and powdered in a blender. 15g of powdered sample without moisture content was elicited in the Soxhlet apparatus with methyl alcohol. The extraction was run

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for a minimum of 20 cycles or till the solvent in the sample container turned colorless (Figure 2).

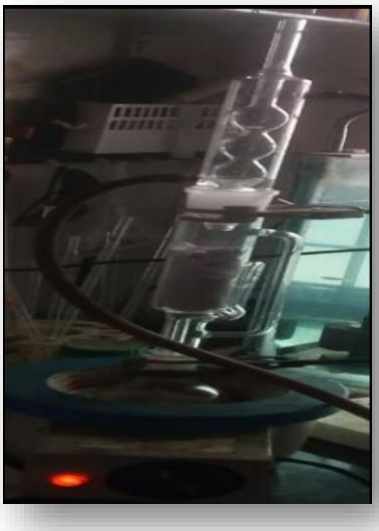

Figure 2. Showing Soxhlet extraction of phytoactives from stem parts of *S. travancoricum*

***In-vitro* alpha-glucosidase inhibition assay**

The alpha-glucosidase inhibition activity was carried out by the method as describe by Kim et al. Briefly, 250µl of the buffer solution was mixed with 50µl of the alpha-glucosidase 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.[12] The inhibition percentage of alpha-glucosidase was evaluated using the formula:

Alpha-glucosidase inhibition (%) = $(A_{Control} - A_{test}) / A_{Control}$ X 100

Where

A, Absorbance

***In-vitro* alpha-amylase inhibition assay**

The alpha-amylase inhibition activity was carried out by the method as describe by Ali et al. Briefly, 120µl of phosphate buffer (pH 7.0) and 60µl of enzyme were added to the test extract of different concentrations. This was incubated at 37°C for 10 minutes. On addition of 250µl of the substrate reagent 2-chloro-4-nitrophenol α-D-maltotrioside (CNPG3), the mixture was again incubated at 37°C for 8 minutes. This was heated on a boiling hot water bath for two minutes so as to arrest the reaction and then cooled. The absorbance was measured at 405 nm.[13] The inhibition percentage of alpha-amylase was evaluated using the following formula.

Alpha-amylase inhibition (%) = $(A_{Control} - A_{test}) / A_{Control}$ X 100

Where

A, Absorbance

RESULTS

The results of effect of stem extract of *S. travancoricum* on alpha-glucosidase inhibition activity was represented in Table 1. Results depicted that the stem extract of *S. travancoricum* at a concentration range of 0.0781µg/mL, 0.1562µg/mL, 0.3125µg/mL, 0.625µg/mL, 1.250µg/mL, and 2.500µg/mL showed inhibition effect of 30.66%, 46.59%, 54.20%, 71.83%, 75.73%, and 82.17% respectively in alpha-glucosidase inhibition activity. The IC_{50} value of stem extract of *S. travancoricum* in alpha-glucosidase inhibition activity was found to be 150.10µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 0.19µg/mL.

Table 1. Effect of stem extract of *S. travancoricum* on alpha-glucosidase inhibition activity

Variables	Conc. (µg/mL)	Inhibition %	IC_{50} (µg/mL)
Control	0.00	0.00	
Acarbose	0.0781	30.66	0.19
	0.1562	46.59	
	0.3125	54.20	
	0.625	71.83	
	1.250	75.73	
	2.500	82.17	
Stem extracts of *S. travancorium*	6.250	10.39	150.10
	12.500	13.09	
	25.000	19.98	
	50.000	31.56	
	100.000	42.13	
	200.000	60.34	

Values were expressed as mean

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The results of effect of stem extract of *S. travancoricum* on alpha-amylase inhibition activity was represented in Table 2. Results depicted that the stem extract of *S. travancoricum* at a concentration range of 0.0781µg/mL, 0.1562µg/mL, 0.3125µg/mL, 0.625µg/mL, 1.250µg/mL, and 2.500µg/mL showed inhibition effect of 30.48%, 46.49%, 54.06%, 71.72%, 75.72%, and 82.12% respectively in alpha-glucosidase inhibition activity. The IC_{50} value of stem extract of *S. travancoricum* in alpha-amylase inhibition activity was found to be 48.38µg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 0.19µg/mL.

Table 2. Effect of stem extract of *S. travancoricum* on alpha-amylase inhibition activity

Variables	Conc. (µg/mL)	Inhibition %	IC_{50} (µg/mL)
Control	0.00	0.00	
Acarbose	0.0781	30.48	0.19
	0.1562	46.49	
	0.3125	54.06	
	0.625	71.72	
	1.250	75.72	
	2.500	82.12	
Stem extracts of *S. travancorium*	6.250	27.68	48.38
	12.500	32.46	
	25.000	47.05	
	50.000	61.64	
	100.000	75.28	

Values were expressed as mean

DISCUSSION

Several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. The greatest bioresource for pharmaceutical intermediates, modern and traditional medicine, nutraceuticals, food supplements, folk remedies, and chemical entities for synthetic drugs is found in medicinal plants.[14] Diabetes is an important human ailment afflicting many from various walks of life in different countries. A more practical approach would involve a series of *in-vitro* prescreens before testing a potential new hypoglycaemic agent in animals. This is because there are no perfect models for type II diabetes, and there are social and financial constraints on the extensive use of animals in experimentation.[15] Furthermore, alpha-amylase and alpha-glucosidase inhibitors have become a new treatment strategy to combat diabetes mellitus,[15] since these enzymes are responsible for digestion of carbohydrates and increasing the postprandial glucose levels in diabetic patients. Inhibiting their activity could help in controlling postprandial hyperglycemia.[8] Therefore, in the current study we aimed for evaluation of *in-vitro* antidiabetic activities of some endangered plant species collected from the western ghats.

Results of our study delineated that The IC_{50} value of stem extract of *S. travancoricum* in alpha-glucosidase inhibition activity was found to be 150.10µg/mL and 48.38µg/mL in alpha-glucosidase and alpha-amylase inhibition activities. These findings implied the stem extracts of *S. travancorium* showed the best antidiabetic activity in both the methods. Hence stem extract of *S. travancorium* will be the best source for antidiabetic activity with higher inhibition activity. Therefore, the *S. travancorium* samples could be very good source in the preparation of antidiabetic drugs.

Literature reports evidenced that plant extracts have been tested with remarkable alpha-glucosidase and alpha-amylase inhibition activities. For instances Vanitha and Keshamma reported the IC_{50} value of 30.56 µg/mL of *aq.* garlic extract in alpha-glucosidase activity in comparison with the standard antidiabetic drug acarbose (41.52 µg/mL).[8] Suchitra and Keshamma found IC_{50} value of 56.24 mg/ml and 32.38 mg/ml for methanolic leaf extract of *A. paniculata* in alpha-glucosidase and alpha-amylase inhibition assays.[15] In another research investigation the anti-diabetic potential of methanolic leaf extract of Aloe vera was demonstrated.[14] Furthermore, Keshamma and Kan Kant Patra found IC_{50} value of 12.28 µg/ml and 14.58 µg/ml for ethanolic extract of cumin seeds, and hence authors recommended that cumin seeds could be employed for the management of Type 2 Diabetes and could be considered for development of natural anti-diabetic drugs.[16]

Earlier studies revealed that inhibition of alpha-glucosidase and alpha-amylase can lower the post absorptive rise in blood glucose and hence can be a better strategy in achieving the glycemic goals in diabetic and borderline prediabetics.[17] In concurrence with previous studies present preliminary pilot study

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revealed that the stem extract of *S. travancoricum* showed potent anti-diabetic properties through inhibition of alpha-glucosidase and alpha-amylase enzyme activities.

CONCLUSION

In conclusion, results of this study portrayed that stem extract of *S. travancoricum* exhibited considerable anti-diabetic properties, and hence this study supports the employment of stem extract of *S. travancoricum* in the development of antidiabetic medicinal drugs.

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