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Research Article

Evaluation of *In Vitro* Antidiabetic Activity of Ethanolic Root Extract of *Cassia fistula* L.

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Abstract

This present study has been undertaken to evaluate the preliminary phytochemical analysis, *in vitro* anti-diabetic activity. The root of the plant extract of *Cassia fistula* L. indicates the presence of secondary metabolites like flavonoids, glycosides were used. The presence of these phytochemicals in high concentration account for the significant hypoglycemic effect of *Cassia fistula* L. It is a tropical herb/frob which has been traditionally used in the management DM. In vitro alpha-amylase enzyme inhibition of Cassia fistula L. extract was evaluated. The study shows significant inhibition activity of ethanolic root extract of *Cassia fistula* L. by using *in vitro* -amylase and glucose diffusion assay, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

Keywords: *Cassia fistula,* anti-diabetic activity, DM (diabetes melllitus)

Article Info

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Contents

1. Introduction

Diabetes mellitus (DM) is a chronic complicated metabolic disorder characterized by increased blood glucose level resulting from the defects in insulin secretion, insulin action, or both. Hyperglycemia is suggested to be one major cause contributing to diabetic complication [1-2]. The incidence of DM is rising at alarmingrate all over the word in the coming years. According to the diabetic atlas of the International Diabetic Federation, 382 million people were affected by diabetes worldwide in the year 2013 and diabetes prevalence is expected to 592 million by the year 2035. The World Health Organization projects that

diabetes will be the7th leading cause of death in 2030 [3-6].Management of diabetes is a global health problemand successful treatment is yet to be discovered. Cur-rently available therapies for diabetes include insulin and various oral antidiabetic agents such as sul-fonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation phytochemicals. Many Indian medicinal plants have been found to be useful in the management of diabe-tes acting though variety of mechanisms. Medicinal plants provide better alternatives as they are lesstoxic, easily available and affordable and many of the currently available drugs have been derived directlyor indirectly from them [7].

2. Materials and Methods

Plant Collection and Identification

Dried entire plant of *Cassia fistula L.* Was collected from Tirumala forest. It was authenticated Dr. K. Madhava chetty, Dept. Of Botany, S V University, Tirupati.

Preparation of Plant Extraction

The plant was shade dried at room temperature and was subjected to size reduction to a coarse power by using dry grinder. 50grams of this coarse power was packed into Soxhlet apparatus and was subjected to extraction sequentially with 500ml of n-hexane, ethyl acetate and ethanol. The extraction was continued until the colour of the solvent in the siphon tube became colorless. Extraction procedure was carried out in RIP NLR. Extracts of ethyl acetate and ethanol were subjected to evaporation by using Rotary evaporator at 60ºc.

***In-Vitro* Anti Diabetic Evaluation**

Glucose Diffusion Assay [8]

Plant extracts were mixed with glucose and placed in the sealed dialysis membrane and kept in the orbit shaker bath at 37ºC, at 150rpm. The movement of glucose across the membrane into the external solution was measured at periodic intervals using commercial GOD-POD kit.

Procedure

Dialysis membrane containing 2ml of 25mM glucose solution was mixed with 1ml of different plants extracts and was placed in the centrifuge tube containing 45ml 0.15MNaCl and then kept in orbit shaker bath at 37ºC at 150rpm. The movement of glucose into the external solution was monitored at set of time intervals using GOD-POD kit. Glucose concentration in the external solution was expected as mg/dl/hr.

Inhibition of Alpha-Amylase Enzyme [9]

Procedure

A starch solution (1%) was obtained by stirring 0.1g of potato starch in 100ml of 16Mm of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5mg of alpha-amylase in 100ml of distilled water. The colorimetric reagents is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96Mm. Both control and plant extracts (200, 400, 600, 800, 1000 and 1200µg/ml) were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3,5 dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540nm.

Calculation

Percentage inhibition (I %) = (Ac- As) / Ac × 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.

3. Result and Discussion

Table 1: Preliminary phytochemical analysis of ethanolic extract of *Cassia fistula* L.

Test	Results
Test for Flavonoids a) Shinado's test b) Sodium hydroxide test	Present Present
Test for Alkaloids a) Dragendroff's test b) Mayer's test c) Wagner's test	Absent Absent Absent
Test for Steroids Salkowskis test	Absent
Test for Tannins With lead acetate	Absent
Test for Saponins: Foam test	Absent
Test for Terpenoids With Tin and thionyl chloride	Absent

Test for Glycosides a) Borntrager's test b) Liermann-burchard's test c) Legal's test	Present Present Present
Test for Carbohydrates Molish's test	Absent

Table 2: Mean glucose intensity in the external solution of various extracts of *Cassia fistula* L. at different time intervals

Time in hours	Control (in the absence of extract)	n-Hexane extract (50mg/ml)	Ethyl acetate extract (50mg/ml)	Ethanol extract (50mg/ml)
1h	134.33±1.20	105.33±1.20[a]	94±1.15[a]	83.33±0.88[a]
3h	201.33±1.76	184±1.15[a]	155.33±0.88[a]	116.33±0.88[a]
5h	244.33±1.76	216±1.15[a]	193.66±0.88[a]	157.33±1.20[a]
24h	312.33±2.02	293±1.15[a]	256.33±1.45[a]	228.66±1.76[a]
27h	316.66±1.76	303.33±1.20[a]	262.33±0.88[a]	232±2.18[a]

*Glucose values are expressed as mean ±SD a -p<0.001- compared to control

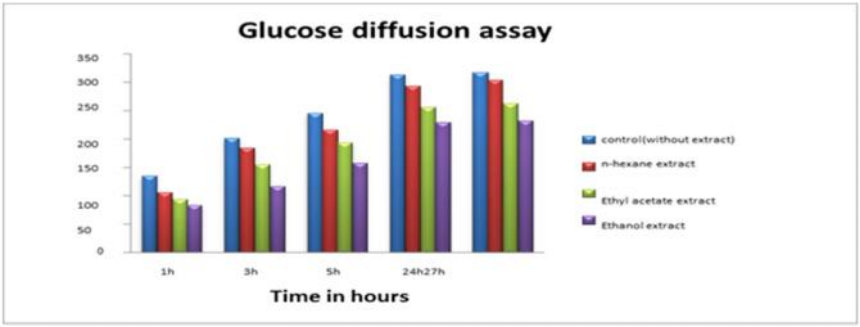

Figure 1: Mean glucose intensity in the external solution of various extracts of Cassia *fistula* L.

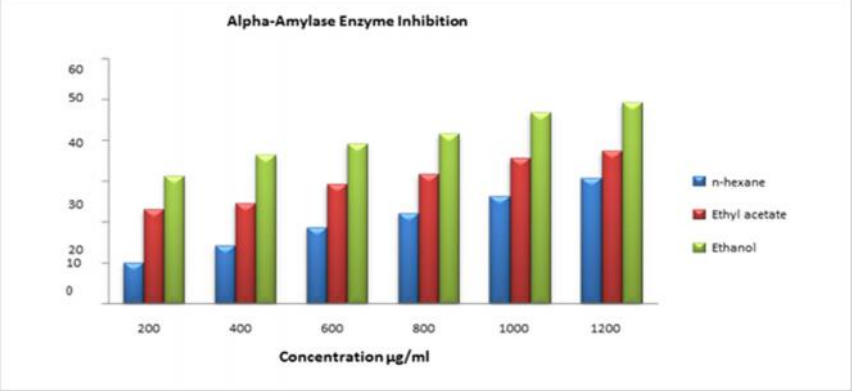

Figure 2: % inhibition of alpha amylase Enzyme

Table 3: *In-vitro* anti diabetic activity of root of *Cassia fistula* L. in alpha amylase enzyme inhibition method

S.No	Concentration of sample (µg/ml)	% Inhibition		
		n-hexane	Ethyl acetate	Ethanol
1	200	10.02	23.09	31.10
2	400	14.21	25.49	36.34
3	600	18.50	29.06	39.09
4	800	22.19	31.67	41.48
5	1000	26.34	35.64	46.71
6	1200	30.63	37.39	49.14

4. Conclusion

The present study we conclude that the preliminary phytochemical analysis of *Cassia fistula L*. indicated the presence of flavonoids, Glycosides. *In vitro* Glucose diffusion properties of *Cassia fistula* L. extract was evaluated using dialysis membrane and GOD-POD kit. *In vitro* alpha-amylase enzyme inhibition of *Cassia fistula* L. extract was evaluated. In this present study was exhibited significant inhibition activity of ethanolic root extract of *Cassia fistula* L. by using *in vitro* α-amylase and glucose diffusion assay, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of anti diabetic agent.

5. Reference

[1] American Diabetes Association (ADA). Diagnosis and classification of diabetesmellitus. Diabetes Care. 2011;34 Suppl 1:62-9.

[2] International Diabetes Federation. Diabetes Atlas. Sixth edition. 2013.

[3] World Health Organization. Global status report on non-communicable diseases2010. Geneva. 2011; pp. 1-176.

[4] Dewanjee S, Das AK, Sahu R, Gangopadhyay M. Antidiabetic activity ofDiospyros peregrina fruit: effect on hyperglycemia, hyperlipidemia andaugmented oxidative stress in experimental type 2 diabetes. Food ChemToxicol.2009;47(10):2679-85.

[5] Saxena A, Vikram NK. Role of selected Indian plants in management of type 2diabetes: a review. J Altern Complement Med. 2004;10(2):369-78.

[6] Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants usedfor diabetes mellitus. J Acute Dis. 2013;2(3):196-200.

[7] Noor A, Bansal VS, Vijayalakshmi MA. Current update on anti-diabetic biomolecules from key traditional Indian medicinal plants. Curr Sci. 2013;104(6):721-7.

[8] Gallagher AM, Flatt PR, Duffy G, Abdel-Wahab YH A. "The effects of Traditional Antidiabetic plants on *in vitro* Glucose Diffusion". Nutrition Research. 2003; 23:43-424.

[9] Manikandan R, Vijaya Aanad A and Durai Muthumani G. "Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of *Psidium guajava* leaves". International Journal of Current Microbiology and Applied Sciences. 2013; 2(2): 15-19.