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Phytochemical Evaluation and *In-vitro* Alpha Amylase Inhibitory Activity on Leaves of *Euphorbia heterophylla* Linn.

S Divya, M Sathish*, V Govindaraj, and M Megala.

Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 600 003, Tamil Nadu, India.

ABSTRACT

Diabetes mellitus is a group of metabolic disorders characterized by high blood sugar levels(glucose) that results from defects in insulin secretion or its action or both. Research work has been made to develop drugs with lesser side effects and good hypoglycaemic activity. Plants have found to possess natural alpha amylase and alphaglucosidase inhibitors which has good hypoglycaemic activity. The aim of the current study is to evaluate the phytochemical properties and *invitro* antidiabetic activity on leaves of *Euphorbia heterophylla.* The ethanol extract of leaves was subjected to preliminary phytochemical screening which showed the presence of glycosides, flavonoids and phenolic compounds. When the extract is separated using HPTLC it was found that the R_f value matched with that of standard marker quercetin. The *invitro* α-amylase inhibition assay showed that there is a dose dependent increase in percentage inhibition when compared to standard acarbose. Thus the antidiabetic activity of the leaves of *Euphorbia heterophylla* may be due to presence of flavonoids.

Keywords: Diabetes mellitus, *Euphorbia heterophylla,* α-amylase , Antidiabetic activity

Corresponding author

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INTRODUCTION

Diabetes mellitus is a chronic disease associated with abnormally high levels of the sugar glucose in the blood. Diabetes is due to inadequate production of insulin or inadequate sensitivity of cells to the action of insulin. The signs and symptoms of both types of diabetes include increased urine output and decreased appetite as well as fatigue. Diabetes is diagnosed by blood glucose testing, the glucose tolerance test, and testing of the level of glycosated haemoglobin. The mode of treatment depends on the type of diabetes. The major complications of diabetes include dangerously elevated blood sugar due to diabetes medications, and disease of blood vessels which can damage the eyes, kidneys, nerves and heart. Blood glucose levels are highly controlled by insulin, a hormone produced by the pancreas. Insulin lowers the blood glucose level. When the blood glucose elevates, insulin is released from the pancreas to normalize the glucose level by promoting the uptake of glucose into body cells. In patient with diabetes, the absence of insufficient production of or lack of response to insulin causes hyperglycemia.[1]

When considering appropriate pharmacologic therapy, a major factor to consider is whether the patient is insulin deficient, insulin resistant, or both. Treatment options can be divided into non insulin therapies- insulin sensitizers, secretagogues, alpha glucosidase inhibitors, incretins and sodium glucose cotransporter 2 (SGLT-2) inhibitors and insulins.[2] The inhibition of alpha glucosidase and alpha amylase, enzymes involved in the digestion of carbohydrates, can significantly reduce the post prandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type 2 diabetic and borderline patients.[3]

Euphorbia heterophylla is a annual branched herb has milky juice and bright red colour. It is originated in the tropical and subtropical regions of America but is now disrtributed throughout tropical Africa, Asia and the Pacific in a total of atleast 65 countries. Leaves contain a red colouring matter, porcetin. Study yielded tannins, phlobatannin, terpenoids, cardiac glycosides, alkaloids and flavonoids. Study yielded terpenoids, quinones,sterol , couramin, starch and protein. The ethno medical uses include purgative action, the hexane, chloroform and ethyl acetate extracts from the roots showed significant antinociceptive activity. The skin irritant, tumor promoting anti-tumor/anti-cancer, antidiabetic and recently anti-HIV activities of Euphorbia species have also been reported in *E.heterophylla*leaf.[4]

MATERIALS AND METHOD

Collection of plant

The leaves of *Euphorbia heterophylla* were collected from Chennai, Tamil Nadu, India. The plant material was identified and authenticated by Botanist Dr.SasikalaEthirajulu, Research officer, CCRAS, Govt.of India, Chennai. The leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40- mesh sieve.

Preparation of Extract

The dried powder of the leaves was extracted by hot continuous percolation method by Soxhlet apparatus, using ethanol as solvent. The extract were concentrated by using a rotary evaporator.[5]

The Preliminary phytochemical screening

The ethanol extract obtained was subjected for phytochemical screening using standard procedure. The dried extract were dissolved in sufficient amounts of respective solvents and tested for various constituents.[6,7].The results of the tests are mentioned in the table

Thin layer chromatography

Flavonoid contents and their presence were determined by the method of Harborne (1998), using quercetin as a standard. The extract was analysed by means of TLC. The ethanol extract was subjected for thin layer chromatography. In solvent system, (n –Butanol- acetic acid – Water) (5:4:1).

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HPTLC Fingerprinting Analysis

The complete CAMAG TLC equipment consists of a fully automatic sample Linomat V sample applicator, a developing chamber. Finally, a Camag TLC scanner is available allowing densitometric evaluation of chromatograms and CATS 4 software for interpretation of data. About 10 mg of ethanolic extract of *Euphorbia heterophylla* was taken and dissolved in respective solvents and the volume was made up to 10 ml in a standard flask (1000µg/ml). Standard (10 mg) was taken and dissolved in methanol. This was transferred into a standard flask and the volume was made up to 100 ml to prepare 100 µg/ml solution. Silica gel 60 F254 and HPTLC aluminium sheets were used as adsorbent (stationary phase). The extracts were applied point-wise from 1000 µg/ml sample solution, 10 µl of the sample was applied on HPTLC aluminium sheets as different tracks in the form of 6 mm wide bands by using a Camag semi-automatic Linomat 5 spotter at a distance of 12 mm. Nitrogen gas was also supplied for simultaneous drying of bands and then using drier for completely drying of bands. HPTLC of different extracts was performed by using the mobile phase: Hexane: Ethylacetate: Methanol [5:4:1]. To saturate the chamber, 10 ml mobile phase was placed in each flat-bottomed Camag twin trough TLC chamber, 30 min before the development of the PTLC plate. The chamber was sealed with parafilm and covered with a steel lid. The developed plates were then dried and scanned using a TLC scanner 3 with Wincats software under 364 nm.[8] All plates were visualized directly after drying and a fingerprint profile was photo documented using a CamagReproster – 3 under 254 nm and 366 nm in UV and visible light. The digital images of the chromatograms were evaluated with the programme CAMAG Video Scan. The captured image was subjected to a visual inspection on the computer screen. The differences found, are specified by the HPTLC system in which the difference is detected and by the R_f value (and colour) of a compound in the system.

***In vitro* anti-diabetic activity**

α-amylase assay:

α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α-amylase solution (0.25 mL) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100 °C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

Inhibition (%) = [(Abs1 − Abs2)/Abs1] × 100 where, Abs1=sample and Abs2 = control

RESULT AND DISCUSSION

PHYTOCHEMICAL SCREENING

Table 1: Phytochemical Screening on leaves of *Euphorbia heterophylla*

S.NO	NAME OF THE TEST	ETHANOL EXTRACT
1	LibermanBurchard (for terpenes& steroid)	_
2	Salkowski (for steroid&terpenes)	_
3	Mayer's (for alkaloids)	_
4	Baljet's test for glycosides	+
5	Legal's test for glycosides	+
6	Test for phenolic ($Fecl_3$)	+
7	Shinoda (for flavanoids)	+

Note: +ve indicates presence, -ve indicates absence

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Table 2: Thin Layer Chromatography on ethanol extract of *Euphorbia heterophylla*

S.No	Solvent System	No of Spots	R_f value
1.	Chloroform: Methanol (9:1)	3	0.28, 0.6, 0.7
2	Hexane: Ethyl acetate: Methanol	2	0.28, 0.7

DISCUSSION

As it was found in Preliminary Phytochemical screening, (Table No.1) the ethanol extract shows the presence of flavonoids, phenolic compounds and glycosides. Table 2, shows thin layer chromatography of the ethanol extract in solvent system used with R_f values. These active constituents may play a major role in the present antidiabetic activity. Herbal principles can be used to develop alternative, inexpensive therapies for treating and managing diabetes. The different modes of action include increase of β-cell stimulation; increase in insulin receptor binding affinity to the released insulin; decrease of hepatic glucose by decreasing gluconeogenesis and glycogenolysis; fighting against free radicals, inhibition of α-amylase and aldose reductase.[9]

HPTLC fingerprinting profile

Authentic marker of flavonol (quercetin) obtained commercially was co-chromatographed. HPTLC chromatogram showed that a maximum number of components were observed under UV and florescence absorbance mode Fig (1) confirmed, the results obtained by TLC performed using the mobile phase:hexane:ethyl acetate:methanol[5:4:1], in which the chromatogram spectrum, clearly showed the R_f values of ethanol extract (0.28) coincided with that of standard quercetin.(0.28). The results are supported by previous reports.[10][11]Putative therapeutic effects of many traditional medicines might be ascribed to the presence of flavonoids.[12]The antidiabetic property of ethanol extract of *Euphorbia heterophylla* leaves may be attributed due to the presence of flavonoid quercetin.

DISCUSSION

By using chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents is not exactly the same for different samples of drug. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug.[13][14]

Figure 1: HPTLC fingerprint analysis of ethanol etract and standard quercetin

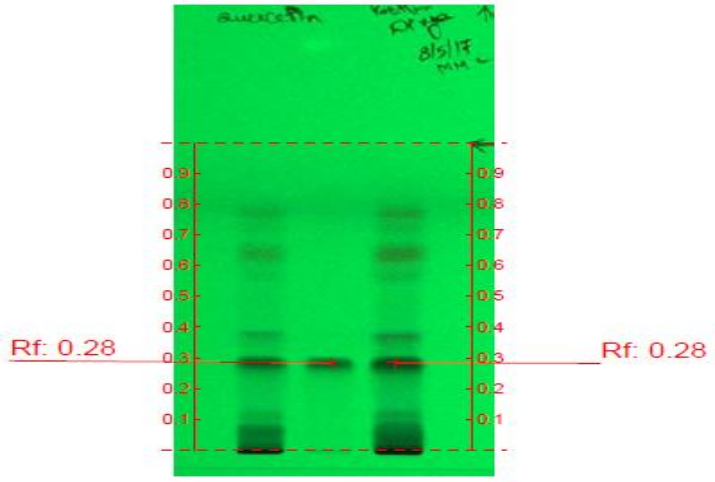

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Table 3: *Invitro* Antidiabeticassay on ethanolicextract of *Euphorbia heterophylla*

COMPOUND	CONCENTRATION (µg/ml)	ABSORBANCE	S-C	S-C/S	%INHIBITION
	50	0.148	0.052	0.35135135	35.13513514
ETHANOL EXTRACT	100	0.176	0.080	0.45454545	45.45454545
	250	0.264	0.168	0.63636364	63.63636364
	500	0.432	0.336	0.7777778	77.7777778
	1000	0.728	0.632	0.86813187	86.81318681
	CONTROL	0.096			
ACARBOSE	50	0.163	0.067	0.41104294	41.10429448
	100	0.212	0.116	0.54716981	54.71698113
	250	0.357	0.261	0.73109244	73.1092437
	500	0.824	0.728	0.88349515	88.34951456
	1000	1.606	1.51	0.94022416	94.02241594
	CONTROL	0.096			

Table 4: Comparison of antidiabetic activity of ethanol extract and standard

Concentration (µg/ml)	Ethanol extract of*Euphorbia heterophylla*	ACARBOSE
100	35.1351	41.10429
200	45.4545	54.71698
300	63.6364	73.10924
400	77.7778	88.34951
600	86.8132	94.02242

From table 3&4 and figure (2) shows clearly that at highest concentration used 1000µg/ml,the percentage inhibition of α-amylase of ethanol extract was found to be 86.813% compared to the standard.α-Amylase hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels.

Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes.Hence ethanol extract of *Euphorbia heterophylla* has good antidiabetic activity by inhibition of α-amylase enzyme.[15]

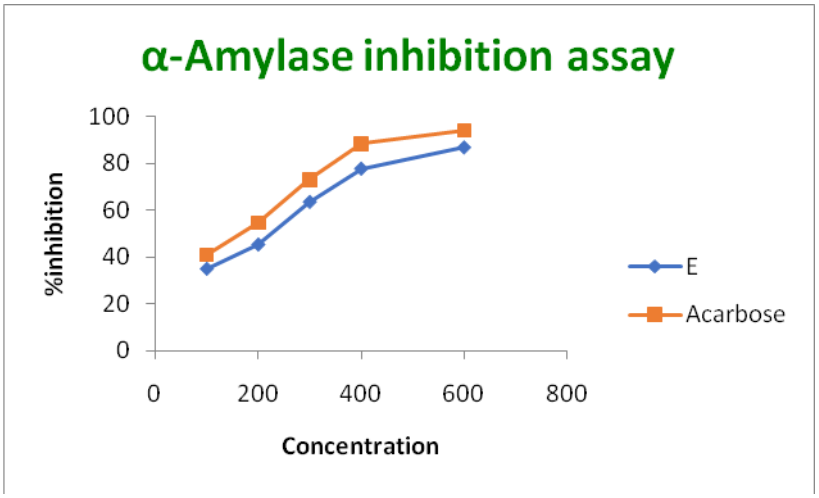

Figure 2: Graphical representation of alpha amylase inhibition assay

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CONCLUSION

The ethanol extract of *Euphorbia heterophylla* is proved to have good antidiabetic activity. The antidiabetic activity may be due to flavonoid quercetin present in the ethanol extract. More research work needs to be done to isolate the active constituent from the plant and evaluate the *invitro*and *invivo* antidiabetic activity.

REFERENCES

[1] http://www.diabetes.org/diabetes-basics
[2] http://clevel andclinicmeded.com/medicalpubs/diseasemanagement/endocrinology/diabetes-mellitus-treatment/
[3] http:// www.ncbi.nlm.nih.gov/pubmed/20470247-alpha amylase inhibitors
[4] Keerthana Kesavan A, Deepa G, Shobana G, Jothi G. Sridharan. International Journal of Pharmacy and Pharmaceutical Sciences 2014;6(8):5- 7
[5] NechetK L, Barreto R W & Mizubuti E S G. Biological Control 2004;30(3):556–565.
[6] Harborne JB. Phytochemical methods 11th ed. New York: In Chapman &, Hall; 1984. 4-5.
[7] Kokate CK. Practical Pharmacognosy. Preliminary Phytochemical Screening. New Delhi: Vallabhprakashan; Chapter 6, p. 106 -111.
[8] Wagner H,Bladt S. Plant Drug Analysis. Second edition. Germany: Springer-Verlag; 2002.
[9] Singh J, Kakkar P. J Ethnopharmacol 2002;123:22-26.
[10] Lakshmi T, Rajendran R, Madhusudhanan N. Int J Drug Dev Res 2012;4:180–185.
[11] Sharma V, Janmeda P, Singh L. Spatulla 2011;1:107–111.
[12] Sharma V, Pracheta. Indian J Nat Prod Res 2012;4(4):348–357
[13] Schultz SC, Bahraminejad S, Asenstorfer RE, Riley IT. J Phytopathol 2008; 156, 1–7.
[14] Liang YZ, XieP, and Chan K. J Chromatogr B 2004;812(1-2):53–70.
[15] Vasei M, Hemmati M, Vessal M. Comp Biochem Physiol C Toxicol Pharmacol 2003;135C(3):357-64.