

In vitro antidiabetic and thrombolytic activity of ethanolic extract of *Phyllanthus acidus* (L) Skeels (EEPA)

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Abstract

Phyllanthus acidus (L). Skeels is a tropical tree belonging to the Phyllanthaceae family. It is commonly known as star gooseberry and it is a highly nutritious edible fruit but also used to treat a wide spectrum of diseases such as inflammatory, rheumatism, bronchitis, asthma, respiratory disorder, hepatic diseases and diabetes in India, Asia, the Caribbean region, and Central and South America. This study aims to determine the *invitro* anti diabetic and *invitro* thrombolytic effect of ethanolic extract of *Phyllanthus acidus*. *Invitro* antidiabetic activities were performed by comparing it with standard (Acarbose). The extract showed comparable α -amylase (IC_{50} 44.38 μ g/ml) and α -glucosidase inhibition activity (IC_{50} 38.29 μ g/ml). The highest concentration of ethanolic extract of *Phyllanthus acidus* exhibited the utmost clot lysis (60.71 %) property compared to standard streptokinase (SK) (64.1%). The present study highlighted that the ethanol extract of *Phyllanthus acidus* has antidiabetic and thrombolytic activities that can be further explored for novel drug development.

Keyword: *Phyllanthus acidus*; Antidiabetic; Thrombolytic; Acarbose; Streptokinase

1. Introduction

Plants are put to medicinal use all over the world since time immemorial. The importance of medicinal plants and traditional health systems in solving the health care problems of the World as gaining increased attention. Due to this resurgence of interest, the research on plants of medicinal importance is growing tremendously. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts and mixtures¹.

Phyllanthus acidus L. Skeels is a tropical tree belonging to the Phyllanthaceae family². Phytochemical studies on the genus *Phyllanthus*, revealed the presence of lignin, terpenes, sterols, polyphenolic compounds, tannins, flavonoids, glycosides and. Biological investigations have also shown antihepatotoxic, antidiabetic, antioxidant, diuretic, anticancer, antimicrobial and anti-inflammatory properties³.

Diabetes mellitus (DM) is commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood⁴. The main disadvantage of currently available drugs is that they have to be given throughout the life and produce side effects. Medicinal plants and their bioactive constituents can be used for treatment of DM throughout the world especially in countries where access to the conventional anti-DM agents is inadequate⁵. A blood clot (thrombus) develops in the circulatory system which consolidates a mechanism in human body to repair the injured blood vessel. If thrombus is formed when it is not needed, this can produce significant

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consequences like embolism, ischemia, heart attack, stroke, and so forth⁶. It is interesting to study the *invitro* antidiabetic and thrombolytic activity on ethanolic extract of *Phyllanthus acidus* L skeels.

2. Material and methods

2.1. Plant collection, authentication and drying

The leaves of *Phyllanthus acidus* (L) Skeels were collected from rural areas of Thrissur, Kerala, India. The specimens collected were identified with the standard literature and authenticated with valid voucher specimens (Voucher no.19385). The plant materials were taxonomically identified by the Botanist, Dr. Madiga Bheemalingappa, Scientist-B, and Department of forest Botany, KFRI, Peechi. The voucher specimen of *Phyllanthus acidus* (L) Skeels was submitted in the Herbarium. The plant material was then dried under shade for about 20 days, powdered with mechanical grinder and stored in an air tight container.



Figure 1 *Phyllanthus acidus*

2.2. Extraction

The plant material was shade dried and coarsely powdered. Around 100g of dried powder was first soaked and defatted with petroleum ether. The dried marc was moistened with the solvent and packed in the Soxhlet extractor and was then extracted by using 1000 ml ethanol for 5 hours. The extract was then filtered through Whatman No. 1 filter paper and concentrated. The extract obtained was then subjected to qualitative and quantitative phytochemical analysis. The percentage yield of extract was found.

2.3. *In vitro* Alpha Amylase Assay

Different concentration of extract (100,200,300,400, 500 μ l) was taken into different test tubes. Made the volume to 0.5 ml with phosphate buffer of pH 6.9; Control was prepared by taking 0.5 ml of phosphate buffer. The solutions were then treated with 0.5 ml of alpha amylase (0.5mg/ml). The solution was incubated at 25 °C for 10 minutes. Added 0.5 ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25 °C for 10 minutes. The reaction was stopped by adding 1.0 ml of DNSA and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Blank was measured by taking 1 ml of phosphate buffer. The absorbance of the solution in colorimeter at 540 nm against blank solution was taken. Standard acarbose was prepared in the same manner at different concentrations and absorbance was measured. The results were calculated and expressed in the basis of percentage inhibition.⁷

$$\text{Percentage inhibition} = \frac{\text{Optical density of control} - \text{Optical density of test} \times 100}{\text{Optical density of control}}$$

2.4. *In vitro* Alpha glucosidase Assay

Different concentration of the sample extract (100,200,300,400,500 μ l) was taken with 0.5 ml of 0.1 M phosphate buffer (pH 6.9) and 0.5 ml of α -glucosidase solution (1Unit/ml/min) and preincubated at 25°C for 5 min. Then, 1 ml of p-nitrophenyl- α -d-glucopyranoside (5 mM) was added and incubated at 25 °C for 10 min. After the incubation period, the absorbance readings were recorded at 405 nm and allegorized to a control that had 0.5 μ l of buffer in place of the

sample. Standard acarbose was prepared in the same manner at different concentrations and absorbance was measured. The results were calculated and expressed in the basis of percentage inhibition ⁸.

$$\text{Percentage inhibition} = \frac{\text{Optical density of control} - \text{Optical density of test} \times 100}{\text{Optical density of control}}$$

2.5. *In vitro* thrombolytic activity of *Phyllanthus acidus*

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 2 ml of blood was transferred to the previously weighted sterile eppendorf tubes and was allowed to form clots. The eppendorf tubes were incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each eppendorf tube having clot was again weighted to determine the clot weight (clot weight= weight of clot containing tube- weight of tube alone.) To each eppendorf tube containing preweighted clot, 100 µl aqueous solutions of different extracts of EEPA was added separately. As a positive control, 100 µl of streptokinase and a negative non thrombolytic control, 100 µl of distilled water were separately added to the control eppendorf tubes. All the eppendorf tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and eppendorf tubes were again weighted to observe the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis is shown below.⁹

$$\% \text{ clot lysis} = \frac{\text{Weight of the lysis clot} \times 100}{\text{Weight of clot before lysis}}$$

3. Results and discussion

3.1. Extractive yield

Percentage yield of extraction of leaves of *Phyllanthus acidus* obtained as tabulated below in the table

Table 1 Percentage yield of extract

Extract	Method of extraction	Extractive yield (%w/w)
Petroleum ether	Cold maceration	0.65
Ethanol	Soxhlet extraction	2.86

The extractive yield of petroleum ether and ethanolic solvent extraction of the plant material was performed by cold maceration and soxhlation and a yield obtained was 0.65 and 2.86 % w/w respectively.

3.2. Alpha amylase inhibitory assay

Table 2 Percentage alpha amylase inhibition of EEPA and Acarbose

Concentration (µg/ml)	EEPA		Acarbose	
	OD at 540 nm	% inhibition	OD at 540 nm	% inhibition
Control	0.93		0.93	
10	0.82	11.82	0.63	32.25
20	0.71	23.65	0.49	47.31
30	0.64	31.18	0.31	66.66
40	0.52	44.08	0.25	73.11
50	0.39	58.06	0.14	84.94
IC ₅₀	44.38		21.73	

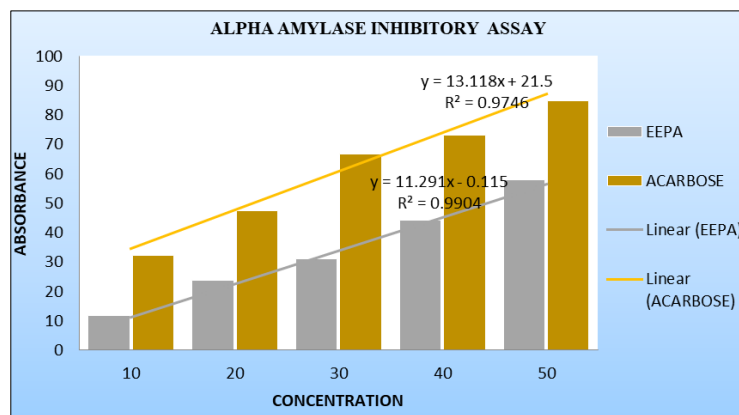


Figure 2 Comparison of IC₅₀ values of EEPA and standard

Inhibition of intestinal pancreatic α -glucosidase and α -amylase activities results in delayed carbohydrate digestion of absorbable monosaccharides leading to a drop in postprandial hyperglycemia. In the present study, the ethanolic extracts of *Phyllanthus acidus* showed α -amylase inhibitory activity. The search for a new α -amylase inhibitor from medicinal plants is a striking method for the management of postprandial hyperglycemia¹⁰. The concentration-dependent α -amylase inhibitory activities and the IC₅₀ values were estimated as indicated in **Figure 2** and **Table 2** respectively. Anti diabetic activity was evaluated by using alpha amylase inhibitory assay and IC₅₀ values for EEPA and Acarbose are found to be **44.38** $\mu\text{g/ml}$ and **24.73** $\mu\text{g/ml}$ respectively.

3.3. Alpha glucosidase inhibitory assay

Table 3 Percentage alpha glucosidase inhibition of EEPA and Acarbose

Concentration ($\mu\text{g/ml}$)	EEPA		Acarbose	
	OD at 405 nm	% inhibition	OD at 405 nm	% inhibition
Control	1.05		1.05	
10	0.93	11.43	0.77	26.66
20	0.75	28.57	0.56	46.66
30	0.63	40.65	0.32	69.52
40	0.54	48.57	0.19	81.90
50	0.35	66.66	0.04	96.11
IC ₅₀	38.29		21.86	

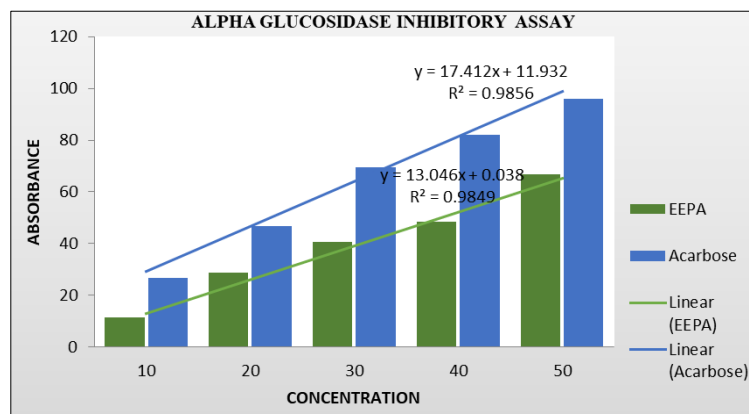


Figure 3 Comparison of IC₅₀ values of EEPA and standard

Intestinal α -glucosidase is a key enzyme for carbohydrate digestion; it has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia. However, mammalian α -glucosidases crude extract from rat intestinal mucosa contains more than one enzyme activity, it is a mixture of sucrase, maltase, isomaltase, glucoamylase, the chromogenic substrate is a common and non-specific substrate, which react indifferently with all these enzymes present in the mixture but inhibition reaction could be more specific and concern only a kind of these enzymes^{11,12}. The concentration-dependent α -glucosidase inhibitory activities and the IC₅₀ values were estimated as indicated in Figure 3 and Table 3, respectively. In the present study, IC₅₀ values for EEPA and Acarbose was found to be 38.29 μ g/ml and 21.86 μ g/ml, respectively.

3.4. *In vitro* thrombolytic activity

Table 4 Determination of percentage clot lysis

Sample	Wt.of empty Eppendroff (W1) gms	Wt.of Eppendroff with clot (W2) gms	Wt of clot (W2-W1) gms	Wt.of eppendroff after clot lysis (W3) gms	Wt.of released clot (W2-W3) gms	% clot lysis
Blank	1.20	1.53	0.33	1.50	0.03	9.09
EEPA (10)	1.22	1.65	0.43	1.47	0.18	41.86
EEPA (20)	1.21	1.77	0.56	1.43	0.34	60.71
STK	1.21	1.60	0.39	1.35	0.25	64.10

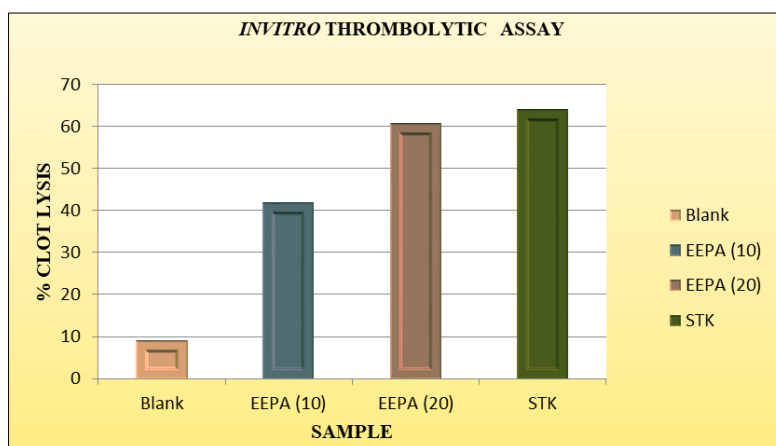


Figure 4 Comparison of percentage clot lysis of EEPA and standard

Thrombosis is the formation of blood clot inside a blood vessel that can block the blood flow in the circulatory system. When a blood vessel is damaged or roughened, the body's defense mechanism uses thrombocytes (platelets) and fibrin to form a blood clot for preventing blood loss. The clotting process starts when the activated platelets form bonds with other platelets in order to form platelet plug and also form bonds with the white blood cells, and form a complex process of clot formation and growth¹³. The lower and higher concentration of ethanolic extract of *Phyllanthus acidus* exerted 41.86% and 60.71% of clot lysis respectively from clotted blood while for standard (streptokinase) and control are 64.10% and 9.09% respectively which are mentioned in Table 4 as well as in the **Figure 4**. So, this draws out that the ethanolic extract *Phyllanthus acidus* possess comparable thrombolytic activity.

4. Conclusion

The plant *Phyllanthus acidus* is tagged with a number of health beneficial uses. This investigation covers metabolic and haematological studies on the leaves of *Phyllanthus acidus*. Phytochemical profiling by GC-MS marked the presence of many active phytochemical constituents which shows the existence of antidiabetic as well as thrombolytic activity. Presence of significant amount of flavonoids and moderate amount of phenols and alkaloids estimated in turns leads to

detailed phytochemical investigation through GC-MS analysis. Totally 21 bioactive constituents were identified. Among the phytochemicals identified, most of them showed antidiabetic as well as *invitro* thrombolytic activity. The present research will enhance the existing knowledge of *Phyllanthus. acidus* and also pave way for more research work to be conducted to unravel the hidden properties of the plant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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