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Research Article

Phytochemical investigation and in vitro anti-diabetic activity of *Terminalia catappa* leaves

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Abstract

Alpha-glucosidases and alpha-amylases play the crucial role in the carbohydrate digestion. The inhibition of these enzymes may be effective in retarding glucose absorption and it can be effectively implement for the management of blood glucose. Conventional knowledge of medicinal plants has constantly guided the search for new cures. In this study we investigate the *in vitro* anti-diabetic effects of methanolic extract of *Terminalia catappa*. The extract exhibit the dosage-dependent increase in inhibitory effect on alpha-glucosidase enzyme (upto 73.2%), and alpha-amylase enzyme (upto 54.04%). The current study supports that the *in vitro* anti-diabetic activity of methanolic extract of *T. catappa* leaves. Further, *in vivo* studies are warranted to confirm the anti-diabetic potentials of *T. catappa*.
Keywords: *Terminalia catappa*; anti-diabetic, glucosidase enzymes

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis, with disorders of various metabolisms resulting from low secretion of insulin or insulin action [1]. About 347 million people have diabetes in worldwide[2]. With a long course and serious complications often ensuing in high death-rate, the treatment of diabetes depleted huge amounts of resources including medicines, diets, physical training and so on. In diabetic patients, medicinal therapy is the unique alternative. Up to now, there are large numbers of chemical or biochemical agents are developed for diabetic patients, but the fact is that there is no one had recovered completely from diabetes.

Scientific records recommended that the natural medicines originated in plants could represent culturally relevant complementary or alternative treatments, as well as this offers good clinical opportunities and also serve in the search for new anti-diabetic agents. *Terminalia catappa* Linn. (*T. catappa*) is found in the warmer areas of India. The different extracts of bark and leaves of the plant have been reported to be hepatoprotective[3], anti-inflammatory[4], anticancer and antioxidant[5] and anti-hepatitis[6]. In type 2 diabetic patients, the inhibition of alpha-glucosidase and alpha-amylase enzymes plays a vital role in the management of post prandial blood glucose level[7]. Thus, objective of the present study is to investigate the phytochemical analysis of various extracts of *T. catappa* and to investigate the *in vitro* anti-diabetic activity of methanolic leaf extract of *T. catappa*.

2. Materials and Methods

2.1 Plant material and extraction

The fresh leaves of *T. catappa* were collected locally and the species was identified and a voucher specimen was deposited at the Rapinet Herbarium St. Joseph's College, Tiruchirappalli, Tamil Nadu. The shade dried pulverized form leaf of *T. catappa* was taken and subjected to successive extraction using methanol and petroleum ether by continuous percolation process in soxhlet apparatus. The aqueous extract was prepared by the maceration with double distilled water. Each extract was concentrated by distilling off the solvent and evaporated to dryness under controlled temperature 40-50°C and the dried extract was kept in air tight containers at room temperature for further analysis. The percentage of yield for methanol extract was found to be 4%.

2.2 Phytochemical Analysis

Freshly prepared extracts of the pulverized leaf were subjected to phytochemical analyses to find the presence of the phytoconstituents such as alkaloids, flavanoids, carbohydrates, tannins, glycosides, phenol, coumarin etc., by standard methods[8].

2.3 *In vitro* methods employed in anti-diabetic studies

2.3.1 Inhibition of alpha-amylase enzyme

A starch solution (0.1% w/v) was prepared 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 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 dinitrosalicylic acid solution 96 mM. The plant extracts 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 dinitrosalicylic acids to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm (Temperature 25ºC±0.1 ºC, pH 4.8; O.D. at 540 nm) [9].

2.3.2 Inhibition of alpha-glucosidase enzyme

Dissolve 25 mg O-dianisidine completely in 1 mL of methanol. Add 49 mL of 0.1 M phosphate buffer (pH 6.5). Then add 5 mg of peroxidase and 5 mg of glucose oxidase to the above prepared O-dianisidine solution. To 0.5 mL of deprotinised plant extract (deproteinization is not necessary in samples with very low protein content) add 0.5 mL distilled water and 1 mL glucose oxidase peroxidase reagent. Into a series of test tubes pipette out 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard glucose solution and make up the volume to 1.0 mL with distilled water. Then add 1 mL of glucose oxidase-peroxidase reagent. Then Incubate all the tubes at 35°C for 40 minutes. Then terminate the reaction by the addition of 2 mL of 6 N-HCl and read the colour intensity at 540 nm[10].

2.4 Statistical analysis

The data are expressed as the percentage of free radical scavenging activity.

3. Results

The results obtained from the phytochemical screening conducted on the *T. catappa* leaf extracts are presented in Table 1. Flavone was absent in all the extracts while tannin and phenol were common to all. The methanol and aqueous extracts contains flavonoids and steroidal glycosides. There was a dosage-dependent increase in percentage inhibitory activity against alpha amylase enzyme. At a concentration of 0.2 ml of plant extract showed a percentage inhibition 8.02% and for 1.0 ml plant extract showed inhibition of 54.04% (Table 2). The *T. catappa* methanol extract revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *T. catappa* extract showed a dosage dependent increase in percentage inhibition. The percentage inhibition varied from 1.02-73.2% for highest concentration to the lowest concentration (Table 3).

Table 1: Phytochemical screening of various extracts of *T. catappa*

SI. No.	Name of the Test	Phytochemical constituents	Methanol Extract	Aqueous Extract	Pet ether Extract
1	Mayer's test Dragondraff test Wagner Test	Alkaloids	+ + -	+ + -	+ + -
2	Molish Test Fehling Test Benedicts Test	Carbohydrates	+ - -	+ - -	+ - -
3	Lead Acetate	Tannins	+	+	+
4	Ferric chloride	Pseudo tannins	+	+	+
5	Ammonia	Chlorogenic acid	-	-	-
6	H_2So_4	Anthocyanin	-	-	-
7	Liebermann's Burchard Test	Steroidal Glycosides	-	-	-
8	H_2So_4	Saponins glycosides	-	-	-
9	Salkowaski test	Steroidal Glycosides	+	+	-
10	Ammonia	Flavonoids	+	+	-
11	Shinoda's Test	Flavones	-	-	-
12	Phenol test	Phenol	+	+	+
13	Sodium chloride	Coumarin	-	-	-

+ : Present - : Absent

Table 2: *In vitro* anti-diabetic activity of *T. catappa* by alpha-amylase method

S. No.	Concentration of Sample (ml)	% of Inhibition
1	0.2	8.02
2	0.4	11.24
3	0.6	26.3
4	0.8	47.14
5	1.0	54.04

Table 3: *In vitro* anti-diabetic activity of *T. catappa* by alpha-glucosidase method

S. No.	Concentration of Sample (ml)	% of Inhibition
1	0.2	1.02
2	0.4	6.09
3	0.6	20.3
4	0.8	47.8
5	1.0	73.2

4. Discussion

The mother globe is a reservoir of innate wherewithal. All through progression, nature provided us with all our desires. Throughout the evolutionary process human was exaggerated by diverse number of illness. Due to mechanical life human has no time to search of leaf and roots of plants of medicinal importance even though they are harmless. Human neglects the medicinal plants around him. There is increasing interest in the use of herbs for the treatment of human diseases. Plants contain a wide variety of compounds that may have biological activities. The present study clearly confirms the presence of various phytoconstituents like alkaloids, tannins, steroidal glycosides, flavonoids and phenol in water, methanol and petroleum ether. These secondary plant metabolites may be responsible for its numerous medicinal effects.

Diabetes mellitus, one of the most frequent endocrine metabolic disorders, has a considerable role on the health and quality of life the patients as well as on the health care system [11]. The large numbers of chemical compounds are identified against diabetes but the complications of

diabetes till continue to be a major problem in the world population. In the digestive organs, alpha-amylase and alpha-glucosidase are the key enzymes which catalyze the final step in the digestive process of carbohydrates. One therapeutic approach results that the reduction of elevation of post prondial blood glucose level by the inhibitory effect of digestive enzymes [12]. Recently, numerous efforts have been made to identify efficient alpha-amylase and alpha-glucosidase inhibitors from medicinal plants for the development of physiologic functional food or lead substances for the treatment against diabetes.

The present results expose that *T. catappa* efficiently inhibits both alpha-amylase and alpha-glucosidase enzymes *in vitro* in a dosage dependent manner. Hence, the inhibition of alpha-amylase and alpha-glucosidase may obstruct the release of d-glucose from dietary complex carbohydrates and which may subsequently delay the absorption of glucose, resulting in reduced plasma glucose levels in postprandial and it may suppress the postprandial hyperglycemia.

5. Conclusion

The present study suggests that the methanolic extract of *T. catappa* leaves inhibits the alpha-amylase and alpha-glucosidase. Further studies needed to elucidate whether *T. catappa* have anti-diabetic potential by *in vivo* models.

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