

In vitro anti-diabetic activity of parasitic plant, *Dendrophthoe falcata* (L.f) Ettingsh

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ABSTRACT

In the present investigation, we have used parasitic plant *Dendrophthoe falcata* (grows on neem) to analyze phytochemicals and evaluated for *in vitro* anti-diabetic activity using four different solvent extracts. The aqueous and methanol extracts yielded saponins, alkaloids, flavonoids, phenolic substances at higher concentration compared to other two extracts. The aqueous and methanol extracts also strongly inhibited the important diabetic enzymes (α -amylase, α -glucosidase, β -glucosidase and sucrase) *in vitro*. Further work is needed to analyse exact antidiabetic compound by separation and biophysical characterization of each compounds.

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KEYWORDS

Dendrophthoe falcata;
Antidiabetic;
Phytochemicals.

INTRODUCTION

The genus *Dendrophthoe* comprises about 31 species spread across tropical Africa, Asia, and Australia^[4] among which 7 species are found in India. *D. falcata* bears grey barks, thick coriaceous leaves variable in shape with stout flowers^[5]. Two of its varieties are widespread in India namely, var. *falcata* (Honey Suckled Mistletoe) and var. *coccinea* (Red Honey Suckled Mistletoe) distinguished by occurrence of white and red flowering, respectively (Flowers of India). Till date, *D. falcata* represents the only known mistletoe with the largest global host range^[8] which is continuously and rapidly widening. *Dendrophthoe falcata* possesses remarkable potentials as a medicinal plant evident from the wound healing, anti-microbial, anti-oxidant, antinociceptive properties of its ethanol extracts^[30,42]. Medicinal properties of this hemiparasite may vary in effects respective to different hosts^[25]. In

the world wide, the incidence of diabetes is increasing; it affects 230 million people of which 30 million are in India. It has been estimated that by the year 2025, the global incidence of diabetes could increase to 350 million. Management of Diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands. The recombinant DNA approach to diabetes management originally considered as a panacea has faced several problems^[23]. It is hypothesized that the ultimate therapy for the type I and II diabetes lies in the herbal approach^[19]. Synthetic drugs are likely to give serious Side effects in addition they are not suitable for intake during conditions like pregnancy^[21,36,48].

Among unexplored parasitic plant *Dendrophthoe falcata* (L.f) Ettingsh (Loranthaceae) occur in India. Angiospermic parasitic plant *Dendrophthoe falcata*, reported to contain biologically active substances such as flavonoid, quercetin^[34], tannins, β -sitosterol, β -amyrin, oleanolic acid^[17,37]. Hence search for a new

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drug with low cost, more potential, without side effects is being pursued in several laboratories all around the world. The present study was aimed at investigatory, the antidiabetic effect of *Dendrophthoe falcata* (L.f) Ettingsh and their phytochemical analysis.

MATERIALS AND METHODS

Collection and processing of plant

On the basis of present literature review and slight experimental modifications, the fresh leaves of *Dendrophthoe falcata* (L.f) Ettingsh (Loranthaceae) growing on the host plant *Azadirachta indica* were collected in the month of April, 2009 during the flowering period at DC Bungalow, Tumkur, Karnataka, India. The plant material was washed with distilled water, shade-air dried ($26\pm 2^{\circ}\text{C}$) and pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and stored in air tight container for further work.

Preparation of plant extracts

25 gm/100ml of powdered leaf was kept for solvent extraction in rotary shaker at 37°C , 72 rpm for 48h. The solvents hexane, ethyl acetate, methanol and distilled water were used with increasing order of their polarity. The solvent extract was then filtered with Whatman No. 1 filter paper and evaporated at a constant temperature of 72°C in hot air oven until a very concentrated extract was obtained. Identification of photochemical constituents such as alkaloids, glycosides, terpenoids, steroids, flavonoids, reducing sugar and tannin were carried out by following standard procedures:

Phytochemical analysis

Phytochemical screening^[41,43,46] Phytochemical screening was performed using standard procedures in the detection of different classes of phyto-constituents like carbohydrates, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, oils and steroids present in the dried leaves of the plant.

Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml

of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for flavonoids

Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was

added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for resins (precipitation test)

About 0.2g of both extracts was washed with about 15ml of 95% ethanol and the mixture poured into 20ml distilled water in a beaker. The formation of a precipitate indicated the presence of resins.

Test for phytosterols (Libermann Burchard's test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for terpenoid and steroid

4.0 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and reddish violet color was observed for terpenoid and greenish blue color for steroids.

In vitro antidiabetic activity

Chemicals and reagents

p-Nitrophenyl- α -d-glucopyranoside, *p*-nitrophenyl- β -d-glucopyranoside, β -glucosidase from almonds and 3,5-dinitrosalicylic acid were purchased from Sisco Research Laboratory, India. A glucose oxidase/peroxidase assay kit was purchased from Aggappe Diagnostics, India. α -amylase (23 u/mg solid) was purchased from Sigma Aldrich, India. All the chemicals and reagents used in the study were of extra pure analytical grade.

Assay of α -amylase inhibitory activity

The effect of *D. falcata* different solvent extracts

on α -amylase activity was studied using an enzyme-starch system^[29]. FRB (1–5%) was mixed by stirring with 25 mL of 4% potato starch in a beaker; 100 mg of α -amylase was added to the starch solution, stirred vigorously, and incubated at 37°C for 60 min. After the incubation period, 0.1M NaOH was added to terminate enzyme activity. The mixture was centrifuged (3000 g; 15 min) and the glucose content in the supernatant was determined.

Assay of α -glucosidase inhibitory activity

α -Glucosidase inhibitory activity was assayed according to the method of Honda and Hara^[16]. Enzyme solution (10 μ L) and varying concentrations of sample emulsion (10–50 μ L) were incubated together for 10 min at 37°C and the volume was made up to 210 μ L with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 μ L of 2 mM *p*-nitrophenyl- α -d-glucopyranoside solution and further incubated at 37°C for 30 min. The reaction was terminated by treating the mixture in a boiling water bath for 5 min. After the addition of 1.0 mL of 0.1 M disodium hydrogen phosphate solution, absorption of the liberated *p*-nitrophenol was read at 400 nm.

Assay of β -glucosidase inhibitory activity

Various concentrations of sample emulsion (10–50 μ L) were pre-incubated with β -glucosidase (5 μ L; 380 U/mL) and the volume was made up to 210 μ L with phosphate buffer (10 mM; pH 7.0). The enzyme reaction was started by adding 200 μ L of *p*-nitrophenyl- β -d-glucopyranoside solution (10 mM) and the mixture incubated (37°C, 30 min). After incubation, distilled water (850 μ L) was added, the solution heated at 100°C for 3 min to stop the reaction, and the absorbance of the solution read at 440 nm^[18].

Assay of sucrase inhibitory activity

The effect of the *D. falcata* different solvent extracts on sucrase activity was assayed according to the method of Honda and Hara^[16]. The enzyme solution (10 μ L) and varying concentrations of sample emulsion (10–50 μ L) were incubated together for 10 min at 37°C and the volume was made up to 200 μ L with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 μ L sucrose solution (60 mM). After 30 min, the reaction was terminated by adding 200 μ L of 3, 5-

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dinitrosalicylic acid reagent and treating the mixture in a boiling water bath for 5 min. The absorbance of the solution was read at 540 nm. The percent inhibitory activities were calculated using the following formula:

$$\% \text{Inhibition} = (\text{Abs Control} - \text{Abs Sample}) \times 100 / \text{Abs Control}$$

Where *Abs control* is the absorbance of the control reaction (containing all reagents except the test sample), and *Abs sample* is the absorbance of the test sample. An untreated enzyme solution was used as the control. All experiments were carried out in triplicate.

Effect of different solvent extracts of *Dendrophthoe falcata* (L.f) Ettingsh in Glucose diffusion

A method described by Gallagher et al.^[11] was used to evaluate the effects of different solvent extracts of *D. falcata* on Glucose movement *in vitro*. This *in vitro* model used consisted of a dialysis tube (6cm X 29.31 mm) (Himedia LA393-5MT-2010) in to which 6 ml of plant extract and 2 ml of 0.15 M NaCl containing 1.65 mM D-glucose were added. The dialysis tube was sealed at each end and placed in a centrifuge tube containing 45 ml 0.15 M NaCl. The tubes were placed on an orbital shaker water bath and incubated at 37°C for 3 h. The movement of Glucose into the external solution was provided. Concentration of Glucose within the dialysis tubing was measured and control tests were conducted in the absence of plant extracts. Glucose concentrations were analyzed by enzymatic method using glucose oxidase kit. All tests were carried out in triplicate and the results were presented as means \pm SD.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical analysis of different solvents yielded different phytochemicals (TABLE 1). Compared to other solvents, the aqueous solvent extracts yielded more compounds. The aqueous extract revealed the presence of carbohydrates, cardiac glycosides, proteins, amino acids, polysterols, alkaloids, phenols, tannins and reducing sugars at higher rate. Whereas, the hexane and ethyl acetate extracts yielded less quantity of phytochemicals. More phenolic substances observed in aqueous extract followed by methanol extract.

Aqueous *D. falcata* extract was demonstrated sig-

nificant inhibitory effects on glucose movement into external solution across dialysis membrane compared to other extracts (TABLE 1). Aqueous extract decreased the overall glucose movement by 46.04 % compared to other extracts methanol (63.13 %), ethyl acetate (89.08 %) and hexane (82.34%). For all solvent extract samples, the overall rates of glucose movement into external solution were higher. Other results are confirmatory with the findings of Gallagher et al.^[11] and Buyukbalci and El^[7], they tried with other different extracts which were most potent inhibitors of glucose movement in the same model system. Gallagher et al.^[11] and Buyukbalci and El^[7] studied the ability to inhibit glucose diffusion using same *in vitro* method with different plant extracts. Gallagher et al.^[11] reported that the plant extracts exhibited a concentration-dependent

TABLE 1 : Phytochemical screening of different extracts from *D. falcata* leaves

Phytochemicals	Extracts			
	Hexane	Ethyl Acetate	Methanol	Aqueous
Carbohydrate	+	+++	+++	+
Cardiac Glycosides	+	++	+++	+
Fixed oils and Fats	--	-	-	-
Proteins and Amino acids	-	-	++	++
Saponins	-	-	-	++
Phytosterol	+	++	+++	+
Alkaloids	-	++	+++	++
Flavonoids	-	+	+++	+++
Phenol	+	+	++	++
Tannins	+	+	++	++
Gums and mucilages	-	-	+	++
Anthraquinones	++	-	-	-
Reducing sugars	-	++	+	+

Note: (-): not detectable. (+): Low quantities. (++) : average quantities. (+++): high quantities. Repeated the each experiment thrice

TABLE 2 : Different solvent extraction of *Dandrophthoe falcata* on diffusion of glucose out of dialysis tube after 3h

Extracts	Glucose diffusion to out of dialysis membrane	Increase of movement (%)
Hexane	82.34 \pm 0.21	30.69 \pm 0.26
Ethyl acetate	89.08 \pm 0.18	31.73 \pm 0.19
Methanol	63.13 \pm 0.23	19.84 \pm 1.02
Aqueous	46.04 \pm 0.31	13.98 \pm 0.24

Each value is the mean \pm standard deviation of three replicate analyses

inhibitory effect on glucose movement. The plants antihyperglycemic activity depends on many factors such as pH, other nutrients and content of meal. Antihypoglycemic activities of most effective plants were in part explained by the ability of the phytochemicals to increase glucose transport and metabolism in muscle and/or to stimulate insulin secretion^[12-15]. Kelble^[1] reviewed that current research on phytochemicals and how they alleviate type 2 diabetes by improving activity in the body. Some antidiabetic plants may exert their action by stimulating the function or number of β -cells and thus increasing insulin release. In some other plants, the effect is due to decreased blood glucose synthesis due to the decrease in the activity of enzymes. In other plants, the activity is due to slow absorption of carbohydrates and inhibition of glucose transport^[10]. In the present study, research has been carried out to evaluate the potential of various extracts which additionally retard the diffusion and movement of glucose^[44].

Effect of phytochemicals on α -amylase

The α -amylase inhibitory activity of different solvent extracts of *D. falcata* was studied using α -amylase starrer model system and the results were presented in Figure 1. α -Amylase inhibitory activity of aqueous extracts is 88% followed by methanol (84 %), ethyl acetate (66 %) and hexane (72 %). From the result it is observed that the aqueous extract increased the α -amylase inhibitory activity and also increased the IC_{50} value and reach statistical significance. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α -amylases play an important role in starch assimilation in human beings and animals, the presence of such inhibitors in food stuffs or plant extracts may be responsible for impaired starch digestion^[27,38]. α -Amylase inhibitors may be of value as novel therapeutic agents^[32]. Generally, inhibition of α -amylase activity by medicinal plants is attributed to several possible factors such as fiber concentration, presence of inhibitors on fibers. Encapsulation of starch and enzyme by the fibers present in the sample possesses reduction in accessibility of starch to the enzyme on fibers leading to decreased amylase activity^[29]. However, inhibition of α -amylase by phytochemicals of plants could be conclusively attributed to the presence of saponins, alkaloids and car-

diac glycosides^[45], flavonoids (Adisakwattana et al., 2010), phenols^[40] and tannins^[28].

Effect on α -glucosidase

All solvent extract treatments significantly inhibited the α -glucosidase (Figure 2). The inhibitory activity of aqueous extract is 72 % followed by methanol (61 %), ethyl acetate (41 %) and hexane extracts (44 %). Similarly β -glucosidase was inhibited by all the different solvent extracts at different range (Figure 3). It is note-

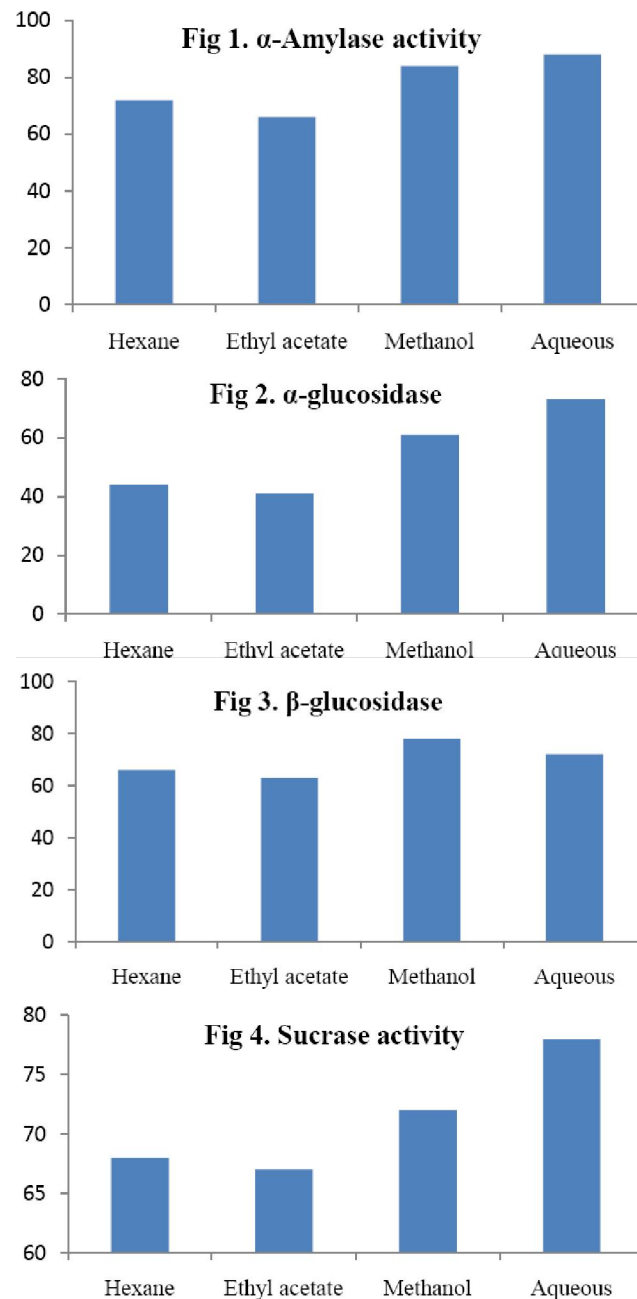


Figure 1 : Effect of *Dendrophthoe falcata* (L.f) Ettingsh on α -amylase, α -glucosidase, β -glucosidase, and sucrase activity

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worthy here that aqueous extract treatment lead to an increase in α -glucosidase. Almost similar result was obtained by some extract against β -glucosidase. Whereas other solvent extracts also less significantly enhanced the inhibitory activity of β -glucosidase. The glucosidase are crucial in many biological process including breakdown of edible carbohydrates^[26], and are also involved in a variety of metabolic disorders such as diabetes^[39]. Thus potent and selective glucosidase inhibitors have many interesting potential applications, especially in diabetes^[39]. α -glucosidase is one among a number of glucosidases located in the brush-border surface membrane of intestinal cells and is a key enzyme of carbohydrate digestion^[9]. α -glucosidase inhibitors block the action of enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides to monosaccharides necessary for gastrointestinal absorption. Postprandial glucose peaks may be attenuated by delayed glucose absorption. The main benefits attributed to α -glucosidase inhibitors are, reduction in both postprandial glycemic levels and the total range of postprandial glucose levels^[47]. The inhibition of α -glucosidase by aqueous and methanol extract of *D. falcata* can be due to the presence of saponins^[24], alkaloids^[49], flavonoids^[31], phenolic compounds^[35] and tannins^[6].

Effect on sucrase activity

Figure 4 clearly shows the effect of different solvent extracts on sucrase activity. More sucrase inhibition was noticed in aqueous extract followed by methanol (72 %), ethyl acetate (67 %) and hexane (78 %). The sucrase inhibition may be due to the presence of phytochemicals, saponins and alkaloids^[20], flavonoids^[2], phenolic compounds^[50] and tannins^[2].

A significant correlation was observed between α -amylase, α -glucosidase, β -glucosidase and sucrase inhibitory activities of aqueous and methanol extracts of *D. falcata*.

CONCLUSION

The result of the present study supports the usage of *D. falcata* in traditional medicine for the management of diabetes and present investigation emphasis that inhibition of carbohydrate hydrolyzing enzymes such as α -amylase, α -glucosidase, β -glucosidase and sucrase

is one mechanism through which *D. falcata* exerts its hypoglycemic effect *in vitro*. It could be used in formulation of functional foods/nutraceuticals for the effective management of diabetes. Furthermore, investigation is required in isolation, identification and structural elucidation of each active phytochemical.

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