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Effect of *In vitro* antidiabetic and anti-obesity activities of hot water extract of *Tephrosia purpurea*

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Abstract
This study evaluated potential antidiabetic and antiobesity properties *in vitro* of selected medicinal plants. The hot water extract of *Tephrosia purpurea* were tested for total antidiabetic capacity using inhibition of alpha- amylase and glusidase capacity assays, followed by anti-obesity activity of inhibition of lipase. Hot water extract of *T. purpurea* had the highest total phenolic content (61.34 µg/ml of GAE/L) and flavonoid content of 46.56 µg/ml Rutin/L) and inhibition of alpha- amylase and alpha-glucosidase (EC_{50} = 62.28 µg/ml) and EIC_{50} = 76.32 µg/ml) respectively compared to acarbose. Similarly, Hot water extract of *T. purpurea* also exhibited significantly lower EC_{50} values for the percentage inhibition of lipase EC_{50} = 61.56 µg/ml compared to orlistate (EC_{50} = 66.98 µg/ml) ($p \leq 0.05$). Phenolic and flavonoid content present in this plants have the potential to use in managing type 2 diabetes and obesity.

Keywords: antidiabetic, antiobesity, *Tephrosia purpurea*

Introduction

Diabetes mellitus is a multifaceted metabolic disorder in the endocrine system characterized by abnormalities in insulin secretion and/or insulin action that leads to enlightened worsening of glucose tolerance and causes hyperglycemia. This ailment is a chief community well-being problematic international and is quickly attractive more common (Adewusi *et al*., 2011) [1]. One healing style to lessening postprandial hyperglycemia is to delay the absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as glucosidase, in the intestine (Chika and Bello, 2010) [2]. The glucosidase enzymes are located in the brush border of the small intestine and are required for the breakdown of carbohydrates before monosaccharide absorption. The alpha-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glycemia and insulin peaks (Patil *et al*., 2012) [6].

Traditional medicine (herbal) is used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them Plant metabolites are potential antidiabetic agents because they apply numerous actions that are both insulinomimetic action and insulin secretagogue. The effects of different classes of flavonoids on glucose control in hyperglycemic rats (glucose overloaded) and in chemically induced diabetic rats. In these studies it was demonstrated that kaempferol-3,7-*O*-(α)-dirhamnoside (kaempferitrin) and kaempferol-3-neohesperidoside act through multiple sites, constituting strong evidence for their insulinomimetic role in assuring glucose homeostasis (Silva *et al*., 2002).

Tephrosia purpurea belongs to family Leguminosae (Sub family-papilionaceae). Leaves imparipinnate; stipules narrowly triangular, 1.5-9 mm x 0.1-1.5 mm; rachis up to 14.5 cm long, including the petiole of up to 1 cm. Flowers are red or purple in leaf opposed racemes, bracteoles usually absent; pedicel 2-6 mm long; flower 4-8.5 mm long, purplish to white. Fruits are large and 2-12 cm long, very densely villous or tomentose. The constituents of Tephrosia purpurea include alkaloids, saponins, glycosides, tannins, flavonoids etc. Some of the constituents may have direct activity and the other inert substances may increase bioavailability and reduces the toxicity. 2 Roots contain tephrosin, dengulin, quercetin, isotephrosin and rotenone. There was no information available in the literature about the *in-vitro* (α-amylase and α-glucosidase inhibitory activity) antidiabetic studies of hot water extract of *Tephrosia purpurea*. Hence, the present study aimed to evaluate α-amylase and α-glucosidase inhibitory activity of hot water extract of *T. purpurea* and its fractions also alkaloids, phenolics, terpenoids, flavonoids, and saponin were identified in the preliminary phytochemical investigation of aqueous extract of hot water extract of *T. purpurea*.

Materials and methods

Plant collection and preparation of extracts

Tephrosia purpurea whole plant was obtained from herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamil nadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-312/2021.

Preparation of hot water extractions

Five grams of freeze-dried samples were extracted in 100 ml of boiling water for 30 min (in triplicates), filtered through Whatman No. 4 paper under vacuum. The samples were reconstituted in dimethyl sulfoxide (DMSO). The supernatants were stored at −80°C until further use for various assays.

Glucose uptake in yeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of Hot water extract of *T. purpurea* (25-100µg/mL) were added to 1mL of glucose solution (20 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). Metronidazole was used as standard drug.

A-amylase inhibitory assay

The pancreatic porcine α-amylase assay was adapted from Sudha *et al*. (2011) [8]. Hot water extract of *T. purpurea* was freeze-dried to obtain a completely dry powder. The test extracts enzyme and soluble starch were dissolved in 20 mM sodium phosphate buffer containing 6 mM NaCl (pH 6.9). The test extracts at different concentrations were dissolved in the buffer solution. To a test tube, 250 µL of pancreatic porcine α-amylase (1 U/mL, dissolved in the buffer (pH 6.9) and 100 µL of test extract at a concentration ranging from 25 to 100 µl/ml were added. The mixture was pre-incubated at 37 °C for 15 min, before the addition of 250 µL of 0.5% starch. The mixture was then vortexed and incubated again at 37 °C for 15 min followed by the reaction termination using 1 mL of dinitrosalicylic acid color reagent. The tubes were placed in a boiling water bath for 5 min, cooled to room temperature and diluted. Two hundred microliters of the reaction mixture were taken into a 96-well clear plate, and the absorbance was read at 540 nm. The control α-amylase at 1 U/mL without any inhibitor represented 100% enzyme activity.

A-glucosidase inhibitory assay

The α-glucosidase inhibitory assay was adapted from Li *et al*. (2010). Briefly, hot water extract of *T. purpurea* at various concentrations prepared in 10 mM potassium phosphate buffer (pH 6.8). To a 96-well clear plate, a reaction mixture containing 20 µL extract at different concentrations, 20 µL α-glucosidase (0.5 U/mL) and 60 µL of 10 mM potassium phosphate buffer (pH 6.8) were pre-incubated at 37°C for 15 min before adding 20 µL of 5 mM p-nitrophenol-α-D-glucopyranoside substrate. The mixture was then incubated at 37°C for the reaction to take place. After 15 min, 80 µL of stop solution containing 200 mM sodium carbonate was added. Then the absorbance at 405 nm was recorded using the microplate reader. The positive control sample was the mixture of the enzyme and substrate without inhibitors. The sample controls and blanks were the mixtures of sample and control, respectively, except α-glucosidase was instead with buffer, respectively. The inhibition (%) of the test sample on α-glucosidase was calculated same way as with α-amylase assay.

Inhibition of lipid peroxidation activity

Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*. (1979). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of the hot water extract of *T. purpurea* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to 1-(E/C) × 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

Pancreatic lipase inhibitory activity

The lipase inhibition activity of hot water extract of *T. purpurea* was determined as per the method proposed by Kim *et al*. (2010). In this assay, the porcine pancreatic lipase activity was measured using p-nitrophenyl butyrate (NPB) as a substrate. Lipase solution (1 mg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0). To determine the lipase inhibitory activity, 1 ml of formulated decoction of cumin seed, flax seed and ragi were pre-incubated with 1 ml of lipase for 10 min at 37°C. The reaction was then started by adding 0.1 mL NPB substrate. After incubation at 37°C for 15 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer and the percentage of inhibitory activity was calculated.

Statistical analysis

All the experiments were performed in triplicates, and complete randomized designs were used. The normality of the residuals was tested using the Anderson-Darling test. The variables were tested using one-way analysis of variance (ANOVA), and the multiple mean comparisons were performed in SAS V8, Cary, NS, USA) with Tukey's test. The data are expressed as a mean ± standard deviation.

Result and discussion

Phytochemical screening

The phytochemical screening of hot water extract of *T. purpurea* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and tannin (Table -1). Previously Kumari, *et al*. (2014) [4] was reported that presence other phytochemicals like flavonoids, alkaloids, carbohydrates, tannins and phenols, gums and mucilage, fixed oils and fats and saponins and lipids.

Table 1: Phytochemical screenings of hot water extract of *T. purpurea*

Sl. No.	Phytochemical Constituents	Observation	Hot water extract of *T. purpurea*
1	Alkaloids -Dragendorff's test -Mayers test	Orange /red precipitate Cream precipitate	+ +
2.	Flavonoids -Alkalai Reagent -Lead aceate test	Intense yellow colour Precipitate formed	+ +
3.	Glycosides -Keller-Killiani test	Pink colour (Ammonia layers)	+
4.	Tannin -$FeCl_3$ test	Blue-black colour	+
5.	Saponins -Frothing test	Foam	-
6.	Terpenoids -Salkowski test	Reddish brown colour ring formed in interface	-
7.	Polyphenols -Ferrozine test	Raddish blue	+
8.	Anthocyanin -Ammonia test	Pink color in ammonia layer	+

+ Positive result; - Negative result

Glucose uptake in yeast cells

The rate of glucose transport across cell membrane in yeast cells system is presented in Fig-1. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in glucose concentrations. The hot water extract of *T. purpurea* exhibited significantly higher activity than at all concentrations. However the highest uptake of glucose was seen in 20mM Glucose concentration.

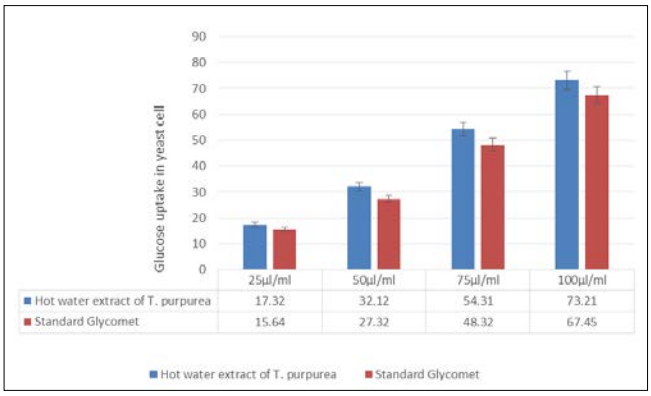

Fig 1: Glucose uptake in yeast cells by hot water extract of *T. purpurea*

Inhibition OF α-amylase

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. In present experimental study it was observed that hot water extract of *T. purpurea* demonstrated inhibition of alpha amylase 78.32%. But the result of hot water extract of *T. purpurea* extract significant inhibition of alpha amylase activity as compared to standard drug glucomet 73.21% (Fig-2). Pavana *et al*., (2009) [7] evaluated the effects of aqueous seed extract of *T. purpurea* on blood glucose and antioxidant status in streptozotocin induced diabetic rats. Hyperglycemia associated with an altered hexokinase and glucose-6-phosphatase activities, elevated lipid peroxidation, disturbed enzymatic Superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic Glutathione, vitamin C and vitamin E antioxidant status were observed in streptozotocin induced diabetic rats..

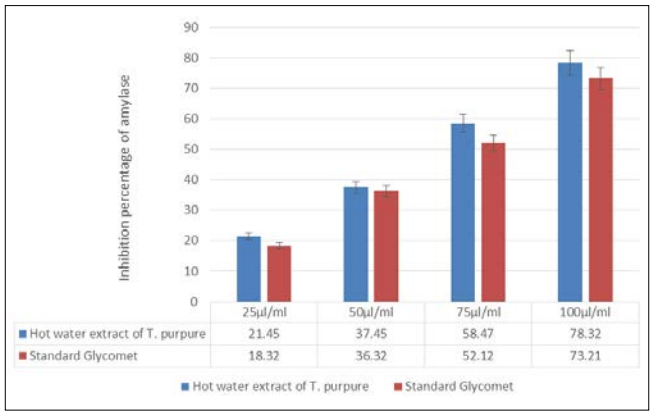

Fig 2: Inhibition of α-amylase by hot water extract of *T. purpurea*

Inhibitory activity of α-Glucosidase

The results of *in-vitro* α-glucosidase inhibitory study are showed in Fig-3. The hot water extract of *T. purpurea* showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 µl/ml tested showed a maximum inhibition of nearly 67.32% hot water extract of *T. purpurea* seems to be less potent in α-glucosidase inhibitory potential compared to glucometers. It may be that α-glucosidase is more sensitive towards glucomet with the concentration required for 50% inhibition (EC_{50}) found to

be 76.32µg/ml. The flavonoid and phenolic acids are known to interact with the enzyme through non-specific binding, leading to inhibition of enzyme activity. The polyphenols related compounds incline to more effective on α-glucosidase inhibition with increase with molecular weight and degree of polymerization (Wang *et al*., 2013) [9].

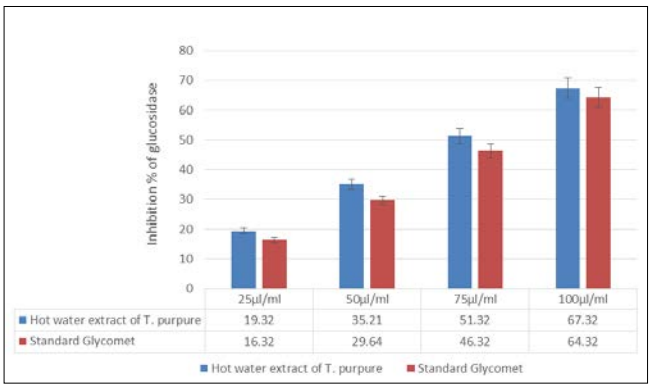

Fig 3: inhibition of α-glucosidase by hot water extract of *T. purpurea*

Inhibition of lipid Peroxidation

The hot water extract of *T. purpurea* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in hot water extract of *T. purpurea* (76.32%) and lowest inhibition percentage of ascorbic acid was found in 72.34% (Table-2). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Table 2: Inhibition of lipid peroxidation activity of hot water extract of *T. purpurea*

Different concentration of extract	Percentage of lipid peroxidation	
	Hot water extract of *T. purpurea*	Standard Vitamin-C
25 µl/ml	16.32±1.78	14.65±1.36
50 µl/ml	35.64±0.89	32.65±0.89
75 µl/ml	52.31±2.36	48.32±2.48
100 µl/ml	76.32±1.24	72.34±0.56
EC_{50} value	67.32	70.23

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Anti-obesity activity inhibition of lipase assay

Result of % lipase inhibition activity of hot water extract of *T. purpurea* was presented in Table 3. Hot water extract of *T. purpurea* showed significantly ($p < 0.05$) higher % lipase inhibition and EC_{50} as compared to standard orlistate, whereas, hot water extract of *T. purpurea* showed significantly ($p < 0.05$) lower % lipase inhibition and EC_{50} 61.56 (Table-3). Hot water extract of *T. purpurea* inhibits the conversion of dietary lipid into fatty acid by hydrolysis. Hot water extract of *T. purpurea* it was reported that flavonoid reduced the triglyceride breakdown and work as a bioactive phytoconstituents. Hot water extracts of *T. purpurea* help in the reduction of gastrointestinal fat digestion and helps in its transit from the body through the feces rather than be absorbed in the body. Hot water extract of *T. purpurea* also contains the flavonoid content which helps in reducing the fat absorption in the body. Obesity is characterized by an increase in fat cells along with an increase in the size of lipid droplets in the cells, as a result of adipogenesis. Therefore, one of the target anti-obesity mechanism is to increase lipolysis and decrease adipogenesis. The influence of native medicinal plant extracts on the regulation of adipogenesis and lipolysis was analysed (Kim *et al*., 2016) [3].

Table 3: Anti-obesity activity inhibition of lipase assay by hot water extract of *T. purpurea*

Different concentration of extract	Hot water extract of *T. purpurea*	Standard Orlistate
25 µl/ml	17.90±0.78	15.78±1.34
50 µl/ml	34.76±2.56	31.45±2.98
75 µl/ml	53.89±2.45	50.82±1.39
100 µl/ml	77.98±0.96	73.56±0.56
EC50 Value	61.56±0.7	66.98±0.7

Results are expressed as percentage inhibited Lipase formation with respect to control. Each value represents the mean+SD of three experiments

Conclusion

On the basis of the results obtained in the present study, it can be concluded that hot water extract of *T. purpurea* exhibited potent antidiabetic and anti-obesity activities. Moreover, hot water extract of *T. purpurea* has been proven through the assessment inhibition of digestive enzyme. Hot water extract of *T. purpurea* also exerted significant lipid peroxidation inhibitory activity of considerable interest. The present finding would be useful for future research directions on the application of traditional medicinal plants in the development of nutraceuticals and pharmaceuticals.

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