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Research Article **Botany**

PHYTOCHEMICAL SCREENING AND ASSESSMENT OF ANTI-DIABETIC ACTIVITY OF *Passiflora foetida* LEAVES

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ABSTRACT

The present study was to investigate the phytochemical screening, histochemical, and evaluation anti-diabetic activity of Passiflora foetida leaves extract. The phytochemical screening Passiflora foetida leaves showed that the presence of tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenol, anthroquinones, glycosides and coumarins while alkaloids was absent in ethanol and aqueous extracts. Quantitative analysis revealed that the *Passiflora foetida* leaves has flavonoids and total phenol. Significant amount of flavonoids (20.05±1.40) and Total phenol (180.00±12.60.). The histochemical analysis further confirmed the flavonoids, tannin, terpenoids, polyphenol and saponin. The ethanolic extract of *Passiflora foetida* showed antidiabetic activity confirmed through inhibition of α-amylase activity and α-glucosidase activity. Overall, it can be concluded the from the present study that *Passiflora foetida* leaves contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals and antidiabetic activity evidence for the relevance of *Passiflora foetida* leaves thus providing scientific validity to its traditional consumption by the local populace of south India. *Passiflora foetida* is a good potential sources for the therapeutic use against diabetic.

Keywords: *Nutritional analysis, Oreochromis niloticus and Catla catla*

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INTRODUCTION

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from Greek (phyton), meaning 'plant'. Some photochemical have been used as poisons and others as traditional medicine. As a term, phyotochemical is generally used to describe plant compounds that are under research with established effects on health and are not scientifically defined as essential nutrients. Regulatory agencies governing food labeling in Europe and the United States have provided guidance for industry limiting or preventing health claims about phytochemicals on food product or nutrition labels. A plant-derived chemical that is not considered an essential nutrient in the human diet but is believed to have beneficial health effects (Akunyili, 2003).

Diabetes is a metabolic disorder of carbohydrate, fat and protein, affecting a large number of population in the world (Pareek et al., 2009). Diabetes mellitus is not a single disorder but it is a group of metabolic disorder characterised by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both.

Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism. When ketones body is present in the blood or urine, it is called ketoacidosis, hence proper treatment should be taken immediately, else it can leads to other diabetic complications (Craig et al., 2009). Diabetes mellitus has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Thevenod, 2008). Diabetes is mainly attributed to the rapid rise in unhealthy life style, urbanization and aging. The present study investigation and evaluation in vitro anti-diabetic activity of *Passiflora foetida* leaves extract.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Passiflora foetida* were collected in 15th month of December 2019 Kunthavai Nachiar College Campus, Thanjavur, Tamil Nadu, India.

Preparation of plant extract:

One gram of the powder of Passiflora foetida leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (ethanol and water). The conical flask containing leaves were shaked it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Quantitative analysis of phytochemicals

Total phenols estimated by the method of Edeoga et al., (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994)

Histochemical tests: (John Peter Paul, 2014; Gersbach et al., 2001).

In vitro α-amylase inhibition study

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis (2007). The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis et al., (2007).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The result was graphically determined by a linear regression method using Ms-Windows based graphpad Instat (version 3) software. Results were expressed as graphically/ mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites. Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. (Sofowara, 1993).

In the present study was carried out on the *Passiflora foetida* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Passiflora foetida* leaves investigated and summarized in Table-1 and figure 2 and 3. The phytochemical screening *Passiflora foetida* leaves showed that the presence of tannins, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenol, anthroquinones, glycosides and coumarins while alkaloids was absent in ethanol and aqueous extracts.

Hassain *et al.* (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. Various organic 11 solvent extracts of *Pedalium murex* were subjected to preliminary phytochemical screenings by Thamizh mozhi *et al.* (2011). Selected 53 traditionally used medicinal plants from western region of India for their qualitative phytochemical screenings, total phenol and flavonoids contents. Pascaline *et al.* (2011) screened phytochemical constituents of some medicinal plants used by the Nandis of South Nandi District, Kenya.

Table.1: Qualitative analysis of Phytochemicals in *Passiflora foetida* leaves extract

S. No	Phytochemicals	Extracts	
		Ethanol	Aqueous
1	Tannin	+	+
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	+	+
5	Terpenoids	++	+
6	Triterpenoids	++	+
7	Alkaloids	-	-
8	Antroquinone	+	+
9	Polyphenol	++	+
10	Glycoside	+	+
11	Coumarins	++	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Quantitative analysis

Quantitative analysis revealed that the *Passiflora foetida* leaves has flavonoids and total phenol. Significant amount of flavonoids (20.05±1.40) and Total phenol (180.00±12.60) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table.2: Quantitative phytochemical analysis of *Passiflora foetida* leaves extract

S. No	Phytochemicals	Results (mg/gm)
1	Total Phenol	180.00±12.60
3	Flavonoids	20.05±1.40

Values are expressed as mean ± SD for triplicates

Falodun *et al*. (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al*. (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* using and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al.* (2007) for its phytochemical properties.

Onwukaeme *et al*. (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. Uma Devi *et al*. (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins (Vaghasiya and Chanda, 2007). Ayoola *et al*. (2008) investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al*., (2009) for its phytochemical and phytochemical properties.

Histochemical studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998). Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan *et al*., 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Table 3 and figure 4 represents histochemical studies of *Passiflora foetida* powder. This study further confirmed the presence of phytochemicals in *Passiflora foetida* .

Table.3: Histochemical studies of *Passiflora foetida* powder

S. No.	Secondary metabolites	Result
1	Polyphenol	++
2	Saponin	+
3	Flavonoids	++
4	Terpenoids	+
5	Tannins	++

(+) Indicates Presence; (++) Moderately present

John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

***IN VITRO* ANTIDIABETIC ACTIVITY**

A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well-conceived as an entity in India. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species (Raghavendra *et al.*, 2010).

The intestinal digestive enzymes alpha-amylase plays a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in management of blood glucose (Latha et al., 2009). The in-vitro α-amylase inhibitory studies demonstrated that *Aloe vera* well anti diabetic activity (Table 4). The percentage inhibition at 100, 200, 300, 400 and 500 µg/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition. *Passiflora foetida* showed a % of inhibition 83.85 % for 500 µg/ml extracts and standard showed inhibition of 94.04%.

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 our experimental study it was observed that ethanolic and aqueous extract of *Passiflora foetida* demonstrated significant Alpha amylase inhibition activity as compared to standard drug acarbose (fig 5).

Table.4: *In vitro* α-amylase inhibition activity of *Passiflora foetida* leaves extract

Concentrations (µg/ml)	% of inhibition	
	Passiflora foetida	**Standard as Acarbose**
100	17.51±0.60	22.09±1.54
200	27.38±1.80	41.05±2.87
300	48.03±2.04	59.01±4.13
400	73.17±2.81	79.84±5.58
500	83.85±3.63	94.04±6.58

Values expressed as Mean ± SD for triplicate

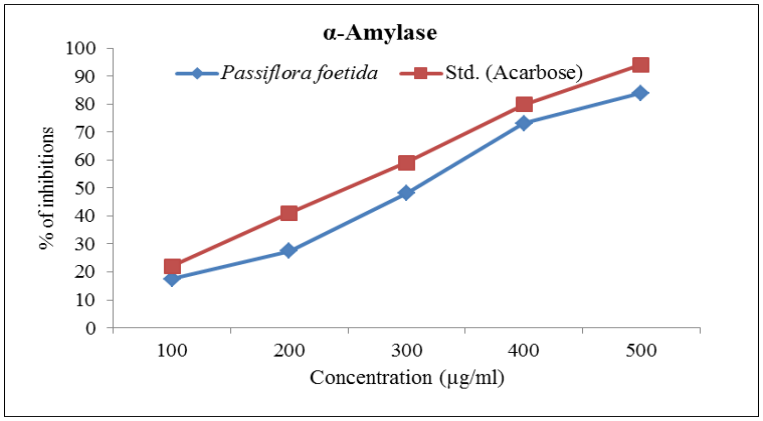

Fig.5: *In vitro* α-amylase inhibition activity of *Passiflora foetida* leaves extract

α-glucosidase catalyzes the final step in carbohydrate digestion which leads to postprandial hyperglycemia. Inhibitors of α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion and causing reduced glucose absorption rate which consequently reduce the postprandial plasma glucose rise (Tarling *et al*., 2008). These inhibitors have been found useful in the control of diabetes mellitus over many years (Layer et al., 1986 Tundis et al., 2010)Many scientists have investigated the plants containing various phytochemicals that exhibit additive and synergistic interaction in antidiabetic properties which exert positive health-promoting effects (Samad et al., 2009). In this present study, *in vitro* α-glucosidase inhibitor activity of ethanolic extract of *Passiflora foetida* was evaluated (table 5 and fig 6). The retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus. The values show that *Passiflora foetida* has 87.14% and standard 90.89%.

Table.5: *In vitro* α-glucosidase inhibition activity of *Passiflora foetida* leaves extract

Concentrations (µg/ml)	% of inhibition	
	Passiflora foetida	**Standard as Acarbose**
100	10.74±0.30	21.76±1.52
200	36.73±0.85	39.07±2.73
300	61.80±1.11	64.43±4.51
400	71.31±1.67	78.09±5.46
500	87.14±1.95	90.89±6.36

Values expressed as Mean ± SD for triplicates

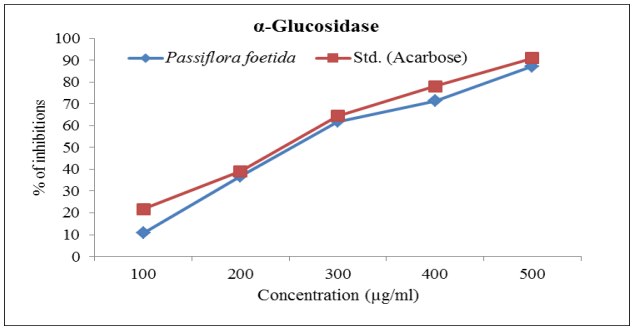

Fig.6: *In vitro* α-glucosidase inhibition activity of *Passiflora foetida* leaves extract

CONCLUSION

Overall, it can be concluded the from the present study that *Passiflora foetida* leaves contains rich source of phytochemicals. This study is the first scientific report that provides convincing phytochemicals and antidiabetic activity evidence for the relevance of *Passiflora foetida* leaves thus providing scientific validity to its traditional consumption by the local populace of south India. *Passiflora foetida* is a good potential sources for the therapeutic use against diabetic.

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