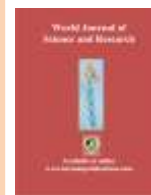


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Research Article

Biochemistry

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTI-DIABETIC ACTIVITY OF *Andrographis paniculata* LEAVES EXTRACT

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, its insulin action and both. It includes a group of metabolic diseases characterized by hyperglycemia. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating etc. Therefore natural alpha amylase inhibitors from the dietary plants can be used as an effective therapy for treating postprandial hyperglycemia with minimal side effects. The medicinal value of the chosen plant *Andrographis paniculata* leaves has not been extensively worked out. Therefore, the present study was to investigate the phytochemical screening, histochemical, fluorescence and antidiabetic activity of *Andrographis paniculata* leaves extract.

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INTRODUCTION

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. Currently available treatment is far from satisfactory and is expensive.

Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes. Keeping this view, the present study aimed to evaluate the *in vitro* anti-diabetic activity of *Andrographis paniculata* leaves. To investigate the phytochemicals in *Andrographis paniculata* leaves. To qualitative analysis of phytochemicals in *Andrographis paniculata* leaves. To quantitative analysis of phytochemicals in *Andrographis paniculata* leaves. To analysis Histochemical changes in *Andrographis paniculata* leaves. To study the *in vitro* antidiabetic activity of *Andrographis paniculata* leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves powder of *Andrographis paniculata* were purchased in December 2018 from siddha medicinal shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract:

One gram of the powder of *Andrographis paniculata* leaves were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (ethanol and water). The conical flask containing *Andrographis paniculata* leaves powder were shake 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). Total phenols estimated by the method of Edeoga *et al.*, (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Saponin determine by the method of Obadoni and Ochuko (2001). Histochemical tests: (John Peter Paul, 2014; Gersbach *et al.*, 2001).

***In vitro* antidiabetic activity**

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). Yeast cells were prepared according to the method of Gupta daksha *et al.*, (2013).

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. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. (Sofowara, 1993).

In the present study was carried out on the *Andrographis paniculata* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Andrographis paniculata* leaves investigated and summarized in Table-1. The phytochemical screening *Andrographis paniculata* leaves showed that the presence of saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenol, anthroquinones, glycosides and coumarins whereas alkaloids was absent in ethanol and aqueous extracts. Tannin present only ethanol extract.

Table 1: Qualitative analysis of Phytochemicals in *Andrographis paniculata* leaves

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

Hassain *et al.*, (2011) screened phytochemical constituents from methanol leaf extract of *Bombax malabaricum*. 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.

Kumar *et al.*, (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained

Table 2: Quantitative phytochemical analysis of *Andrographis paniculata* leaves extract

S. No	Phytochemicals	Results (mg/gm)
1	Flavonoids	100.00 ± 7.00
2	Saponin	100.00 ± 7.00
3	Phenol	181.81 ± 12.72

Values are expressed as mean ± SD for triplicates

Leo Stanley *et al.*, (2011) reported that leaves of *C. pedata* showed the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. Rajmohanan *et al.* (2014) investigated the preliminary phytochemical analysis of various extracts of leaves of *C. pedata* and showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al.*, (2009) for its phytochemical and phytochemical properties.

Histochemical studies

Table 3: Histochemical studies of *Andrographis paniculata* powder

S.No.	Secondary metabolites	Observation	Result
1	Polyphenol	Blue green/Red	+
2	Saponin	Yellow	+
3	Flavonoids	yellow	+
4	Steroids	Blue(or) Green	+

(+) Indicates Presence

John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). 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

glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

Quantitative analysis

Quantitative analysis revealed that the *Andrographis paniculata* leaves has flavonoids, saponin, and phenol. Significant amount of flavonoids (100.00mg/gm), saponin (100.00 mg/gm), and phenol (181.81 mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

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 and Krishnan *et al.*, 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. Table 3 represents histochemical studies of *Andrographis paniculata* powder. This study further confirmed the presence of polyphenol, saponins, flavonoids and steroids in *Andrographis paniculata*.

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. The percentage inhibition at 100, 200, 300, 400 μ g/ml concentration of crude plant extracts shown concentration dependent reduction in percentage inhibition. *Andrographis paniculata* showed a % of inhibition 77.53% for

400 µg/ml extracts and standard showed inhibition of 85.31%.

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield

glucose and maltose. In our experimental study it was observed that ethanolic and aqueous extract of *Andrographis paniculata* demonstrated significant Alpha amylase inhibition activity as compared to standard drug acarbose (Table 4).

Table 4: In vitro α-amylase inhibition of *Andrographis paniculata*

Concentrations	<i>Andrographis paniculata</i>	Standard Acarbose
	% of inhibition	
100µg/ml	40.86±0.98	43.61 ± 0.74
200µg/ml	48.46±0.80	57.23±0.98
300µg/ml	70.26±0.01	74.65±1.67
400µg/ml	77.53±0.90	85.31 ± 1.88

Values expressed as Mean ± SD

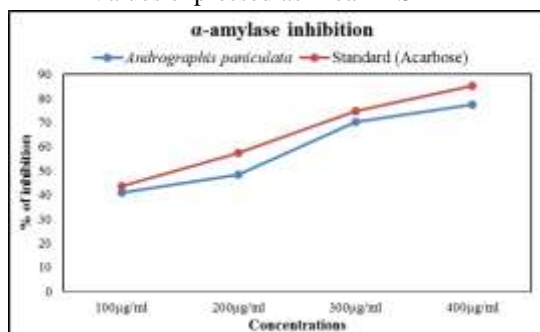


Figure 1: In vitro α-amylase inhibition of *Andrographis paniculata*

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). 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 *Andrographis paniculata* was evaluated. 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 *Andrographis paniculata* has 78.12% and standard 85.69%

Table 5 In vitro α-glucosidase inhibition of *Andrographis paniculata*

Concentrations	<i>Andrographis paniculata</i>	Standard Acarbose
	% of inhibition	
100µg/ml	29.56±1.94	35.35 ± 1.43
200µg/ml	48.84±0.82	59.15 ± 1.41
300µg/ml	65.90±2.19	70.55 ± 1.64
400µg/ml	78.12±1.63	86.69±1.72

Values expressed as Mean ± SD

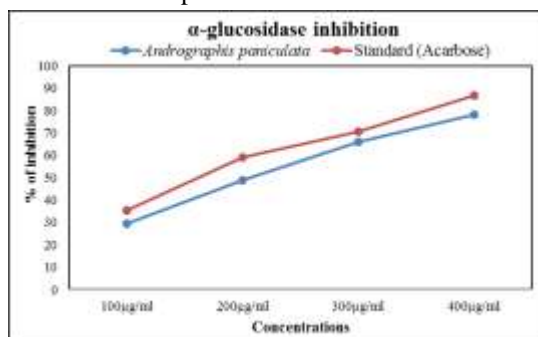


Figure 2: In vitro α-glucosidase inhibition of *Andrographis paniculata*

The rate of glucose transport across cell membrane in yeast cells system is presented in Table 6. In Yeast (*Saccharomyces cerevisiae*), glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterized by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these plant extracts, the glucose uptake was found to increase in a dose dependent manner. The increase in glucose uptake by the yeast cell at

different glucose concentrations i.e. 100, 200, 300, 400 µg/ml respectively. The ethanolic extract of *Andrographis paniculata* exhibited significantly higher activity at all glucose concentrations showing the maximum increase in 400 µg/ml Glucose concentration. Results also indicated that *Andrographis paniculata* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin.

Table 6: Glucose uptake in Yeast cells of *Andrographis paniculata* leaf extract treated

Concentrations	<i>Andrographis paniculata</i>	Standard Metformin
	% of inhibition	
100µg/ml	8.34±6.02	17.32 ± 1.32
200µg/ml	33.33±2.88	40.65 ± 2.28
300µg/ml	50.00±2.88	61.56 ± 2.91
400µg/ml	66.67±2.88	72.75 ± 3.02

Values expressed as Mean ± SD

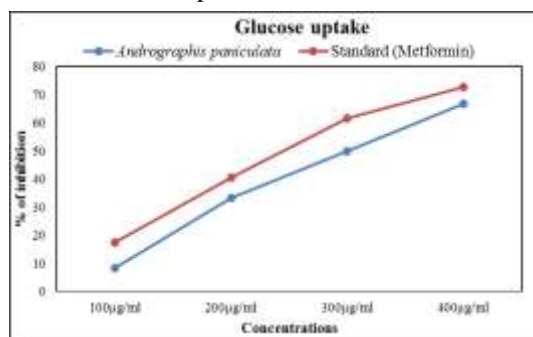


Figure 3: Glucose uptake in Yeast cells of *Andrographis paniculata* leaf extract treated

CONCLUSION

Overall, it can be concluded from the present study that *Andrographis paniculata* 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 *Andrographis paniculata* leaves thus providing scientific validity to its traditional consumption by the local populace of south India. *Andrographis paniculata* leaves extract had a good potential for therapeutic use against diabetic.

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