

## RESEARCH ARTICLE

PHYTOCHEMICAL EVALUATION AND IN VITRO ANTIDIABETIC EFFICIENCY OF ISOPROPANOLIC LEAF EXTRACT OF *PIMENTA RACEMOSA*Anitha, T.A.<sup>1,\*</sup>, Pakutharivu, T.<sup>1</sup>, Nirubama, K.<sup>2</sup> and Akshaya, V.<sup>3</sup><sup>1</sup>Department of Biochemistry, MGR College, Hosur - 635130, Krishnagiri, Tamil Nadu, India.<sup>2</sup>Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.<sup>3</sup>Department of Biochemistry, Government College of Arts and Science for Women, Krishnagiri, Tamil Nadu, India.

## ABSTRACT

The traditional herbal medicines are mainly obtained from plants are used in the management of Diabetes mellitus. The main objective of this work was to assess the presence of phytochemical compounds and to evaluate the *in vitro* antidiabetic activity of isopropanolic extracts of *Pimenta racemosa* leaves by studying their  $\alpha$ -amylase inhibitory activity and glucose transport across yeast cells. Screening of phytochemicals showed positive results for alkaloids, steroids, cardiac glycosides, terpenoids, reducing sugars, anthraquinones, and results of *in vitro*  $\alpha$ -amylase inhibitory studies demonstrated there was a dose-dependent increase in percentage inhibitory activity by the isopropanolic leaf extracts of *Pimenta racemosa*. At a concentration of 1 mg/ml, the extract showed a percentage inhibition 33.6 and for 5 mg/ml it was 91.2. The glucose uptake study was also studied through yeast cells by analyzing the amount of glucose remaining in the medium after a specific time intervals. It serves as an indicator for the capability of isopropanolic leaf extracts of *Pimenta racemosa* to transport the glucose into yeast cells. As a result, we found that the isopropanolic leaf extract of *Pimenta racemosa* have inhibitory activity against  $\alpha$ -amylase and also, which is efficient in glucose uptake. This therapeutic potentiality of *Pimenta racemosa* could be exploited in the treatment of Type 2 Diabetes mellitus. Further studies are also required to elucidate whether the plant have antidiabetic potential by *in vivo* for corroborating the traditional claim of the plant.

**Keywords:** *Pimenta racemosa*, phytochemicals,  $\alpha$ - amylase, glucose uptake, *in vitro* antidiabetic activity.

## 1. INTRODUCTION

Phytochemicals are chemicals of plant origin [1]. They are produced through primary or secondary metabolism of plants [2]. They generally have biological activity in the plant, and play a role in plant growth or defense against competitors, pathogens, or predators [3]. Their easy availability, the least side effects, and low cost of making the herbal preparations make them key player of all available therapies, especially in rural areas. Every plant has a habitat and, is restricted to particular are as on the globe. To make help in every part of the world, there is a need for exploring the antidiabetic potential of an explored plant as well [4].

However, the phytoconstituents responsible for the antidiabetic activity has not been fully identified. However, search for new antidiabetic drugs from plants be still attractive as thus contain a number of natural compounds like glycosides, alkaloids, terpenoids, flavonoids, carotenoids which demonstrate alternative and safe effects on Diabetes mellitus [5]. Phytochemicals are studied by first extracting and isolating compounds from the plant followed by defining their structure or testing in laboratory

model systems, such as cell culture, *in vitro* experiments or *in vivo* studies using laboratory animals [6].

Diabetes mellitus is a major endocrine disorder, in which a person has high blood sugar, either because the pancreas does not produce enough insulin or because cells do not respond the insulin that is produced. It is also supposed to be group of metabolic disorder caused by the failure of the body function properly through carbohydrate metabolism as well as change in lipid and proteins metabolism thus contributing hyperglycemia; increased blood sugar level above normal and glycosuria. Both women and men can develop diabetes at any age [7].

Type 1 Diabetes, Insulin dependent or juvenile onset diabetes mellitus is due to pancreatic islet of destruction predominantly by an autoimmune response. Type 2 Diabetes, it is formerly known as non-insulin dependent diabetes mellitus. It is due to decrease number of receptor, loss of beta cells responsiveness to glucose leading to slow or insulin release by the pancreas. Gestational Diabetes is a form of high blood sugar affecting pregnant women [8]. According to international diabetes and its complications, in 2019 the incidence of Diabetes crossed 366 million

with an estimated 4.6 million death every year. India is one of the prominent diabetic capital among other countries [9].

Diabetes mellitus is now life threatening throughout the world disorder with increasing incidence. Diabetes mellitus is affecting around 25% of the world population of both developed and developing countries. India is 70 million persons and USA is 3.40 million persons suffering from diabetes [10]. Untreated diabetes can cause many complications like renal failure, diabetic retinopathy, and cardiovascular disease atherosclerosis [11]. Antidiabetic drugs are type of medicine which is used to control the increased blood glucose level in the body [12].

The treatment for diabetes has spent vast amount of medicine, diet, physical training and, so on in all countries and, also searching for natural and synthetic compounds overcome diabetic problems [13]. Synthetic drugs are likely to give serious effect in addition they are not suitable for intake during condition like pregnancy [14]. According to World Health Organization (WHO) up to 80% of the population in developing country uses plants, and its product as a traditional medicine for Primary Health Care needs. The WHO has listed 21000 plants which used for medicinal purposes around the world. Among all, 25,000 species are in India. There are about 800 plants which are reported to show antidiabetic potential [15].

Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels [16]. Several species of herbal drugs with potential antidiabetic activity has been described in the scientific literature. Herbal drugs are prescribed due to their good effectiveness, less side effects in clinical experience and relatively low costs [17].

*Pimenta racemosa* is considered as traditional medicinal plant based on its biochemical features and, secondary metabolite product. Secondary metabolites are a varied group of organic compounds produced by plants as establishment of a defense mechanism and interaction with biotic environment. Most of the secondary metabolites are classified according to different biological activities, biosynthetic source, and are used as agro chemicals, bio pesticides, pharmaceuticals food additives, colours, flavours fragrances [18].

Medicinal and natural herbal plant products are traditionally used from long time in many countries for the treatment of Diabetes mellitus. The aim of the present work was to screen the presence of phytochemical compounds and assess the *in vitro* antidiabetic activity of isopropanolic extracts of *Pimenta racemosa* leaves

by studying their effects of  $\alpha$ -amylase inhibitory activity and glucose transport across yeast cells.



**Fig. 1. *Pimenta racemosa***

## 2. MATERIALS AND METHODS

### 2.1 Collection and preparation of plant extracts

The leaves of *Pimenta racemosa* was collected from Krishnagiri District, Tamilnadu, India. The collected leaves are cleaned and dried for 5 to 6 days without sun light. And then made course powder of leaves with the help of dry mechanical grinder and passed through sieve. The powdered leaves were extracted using soxhletion method and defatted with isopropyl alcohol at 30 °C. Extracts were evaporated to dryness and preliminary phytochemical screening was carried out by using standard methods.

### 2.2. Chemicals

Basic bismuth nitrate, Potassium iodide, Acetic acid, Picric acid, Magnesium powder, Acetic anhydride, Conc. Sulphuric acid, Glacial acetic acid, Ferric chloride, Chloroform, Antimony trichloride, Potassium ferric cyanide, Ferric chloride, Hydrochloric acid, Lead acetate, Ammonia solution, Sodium hydroxide, Isopropyl alcohol, yeast cells, Glucose, DNSA (dinitro salicylic acid), Starch, Amylase, Dextrin, Maltose, Phosphate buffer, Sodium chloride, Sodium potassium tartarate, Sodium hydroxide, Disodium hydrogen phosphate were obtained from Himedia (Mumbai, India). All other chemicals used were of analytical grade.

### 2.3. Phytochemical Analysis

Phytochemical screening of the leaves of *Pimenta racemosa* was carried out by the standard protocols [19]. The isopropyl alcoholic extract of plant leaves were screened for the presence of alkaloids, flavonoids, phenols, carbohydrates, amino acids, proteins, and tannins.

### 2.4. In Vitro Antidiabetic Activity

#### 2.4.1. $\alpha$ -Amylase Inhibitory Assay

An extract solution (1% w/v) was prepared by stirring 1g extract in 100ml of 20mM of phosphate buffer (pH 6.9) containing 6.7 mM

sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic  $\alpha$ -amylase (PPA) in 100ml of 2mM phosphate buffer (PBS, pH 6.9) containing 6.7 mM of sodium chloride. 200  $\mu$ l of (2, 4, 8, 10, 15  $\mu$ g/ml) plant extract, 200  $\mu$ l porcine pancreatic  $\alpha$ -Amylase were added and the mixture was incubated at 37 °C for 20 minutes. To the Reaction mixture 100  $\mu$ l 1 % extract solution was added and incubated at 37 °C for 10 minutes. The reaction was stopped by adding 200  $\mu$ l of Dinitro salicylic acid (1g of DNSA, 30g of sodium potassium tartarate and 20 ml of 2 N sodium hydroxide was added, and make up to the final value of 100 ml with distilled water) kept in a boiling water bath for 5 minutes. The reaction mixture was diluted with 2.2 ml of water and absorbance was read at 540 nm.

For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 $\mu$ l distilled water. Control, representing 100% enzyme activity was prepared in a similar way without extract. The experiments were repeated thrice using the same protocol [20].

#### 2.4.2. Glucose Uptake by Yeast

Commercial baker's Yeast was washed by centrifugation (3,000 $\times$ g; 5 mins) with distilled water until the supernatant fluids were clear and a 10%(v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg) were added to 1ml of glucose solution (1gm / ml) and incubated together for 10 mins at 37 °C. Reaction was started by adding 100 $\mu$ l of yeast suspension, vortex and further incubated at 37°C for 60 mins. Then, the tubes were centrifuged (2,500 $\times$ g, 5min) and glucose was estimated in the supernatant by DNSA method. All the experiments were carried out in triplicates [21].

#### 2.5. Statistical analysis

Each experiment was performed in triplicates. The results of amylase inhibitory assay were expressed as mean $\pm$ standard deviation (SD).

### 3. RESULTS AND DISCUSSION

The dried leaves of *Pimenta racemosa* were extracted with isopropanol by soxhlet method. The obtained extracts were subjected to phytochemical screening for its constituents by standard methods, and *in vitro* antidiabetic activity was determined by  $\alpha$ -amylase inhibitory assay and glucose uptake assay by yeast cells.

#### 3.1. Preliminary qualitative phytochemical analysis

The present study revealed that the isopropanolic leaf extracts of *Pimenta racemosa* contained alkaloids, steroids, cardiac glycosides,

terpenoids, reducing sugar and triterpenoids (Table 1).

The preliminary phytochemical screening tests may be useful in the detection of the bioactive phytocompounds may lead to the discovery and development of drugs. These bioactive phytocompounds are reported to have many biological and therapeutic properties [22]. Hence, these results revealed that the isopropanolic leaf extracts of *Pimenta racemosa* is likely to have many medicinal uses.

#### 3.2. Evaluation of *In Vitro* $\alpha$ -Amylase Inhibitory Activity

As shown in the Table: 2 and Figure 2, the *in vitro*  $\alpha$ -amylase inhibitory studies demonstrated there was a dose-dependent increase in percentage inhibitory activity by the isopropanolic leaf extracts of *Pimenta racemosa* against  $\alpha$ -amylase enzyme. At a concentration of 1 mg/ml, the extract showed a percentage inhibition 33.6 and for 5 mg/ml it was 91.2. In this study, isopropanolic leaf extracts of *Pimenta racemosa* showed appreciable  $\alpha$ -amylase inhibitory effects. It may be due to the presence of more chemical constituents such as, flavonoids, terpenoids, and alkaloids in the isopropanolic extracts. This plant-based  $\alpha$ -amylase inhibitor may offer a prospective therapeutic approach for the management of diabetes.

In human digestive system, there are many enzymes in which  $\alpha$ -amylase hydrolyses  $\alpha$ -bonds of polysaccharides into monosaccharide such as starch and glycogen to yield high level of glucose. These glucose units only can be absorbed through intestine and increase the postprandial blood glucose level [23].  $\alpha$ -amylase inhibitors bind to  $\alpha$ -bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide [24]. The inhibition activity of  $\alpha$ -amylase and alpha glucosidase would delay the degradation of carbohydrates, which cause a decrease in the absorption of glucose, resulting in reduction of postprandial blood glucose level [25].

#### 3.3. Glucose uptake study by Yeast Cells

Table 3 reveals the increase in percentage inhibition of glucose uptake by yeast cells with extract concentration ranging from 1 mg/ml to 5 mg/ml. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration i.e. 1 mg/ml the increase in percentage inhibition was somewhat linear but as concentration increases higher till 5 mg/ml it tends to become somewhat more exponential. Glucose uptake in yeast cells is rapid and occurs down the concentration gradient. Phosphorylation accompanies by glucose entry into the cell. Glucose transporters are stereospecific for certain hexoses and carries glucose, fructose, and mannose [26].

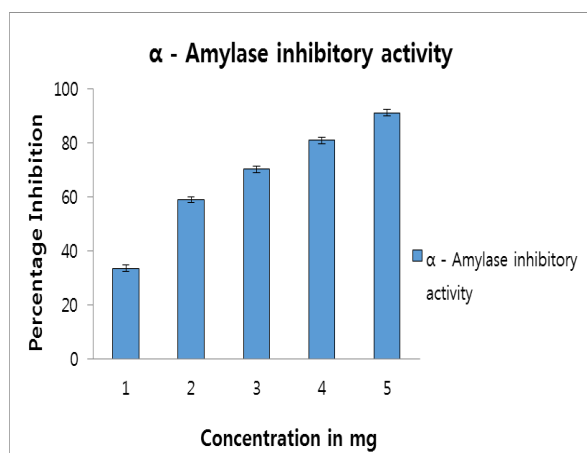
**Table 1. Represents the Quantitative analysis of phytochemicals**

Phytochemicals	Test name	Observation	Inference
Alkaloids	Wagner's test	Reddish brown precipitate	Presence
Steroids	Lieberman's Burchard test	Upper layer turned red, Sulfuric acid layer turned green	Presence
Cardiac glycoside	Killer killani test	Green colour	Presence
Terpenoids	Salkowski test	Yellow colour rings turns reddish brown colour	Presence
Triterpenoids	Antimoni trichloride test	Pink colour	Presence
Reducing sugar	Benedict's test	Brick red	Presence
Anthroquinones	Borutrager's test	Deep red	Presence

**Table 2. The Effect of isopropanolic leaf extract of *Pimenta racemosa* on  $\alpha$ -amylase inhibitory activity**

Concentration of extract (mg/ml)	Percentage Inhibition
1	33.6±0.14
2	59.0±0.07
3	70.3±0.12
4	81.0±0.06
5	91.2±0.13

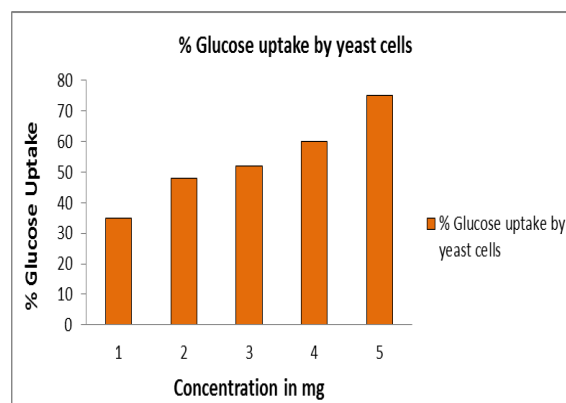
**Fig. 2. The effect of isopropanolic leaf extract of *Pimenta racemosa* on  $\alpha$ -amylase inhibitory assay**



**Table 3. reveals Effect of isopropanolic leaf extract of *Pimenta racemosa* on glucose uptake by yeast cell**

Glucose uptake by yeast cell (concentration in mg)	% of Glucose uptake
1	35
2	48
3	52
4	60
5	75

**Fig. 3. The effect of isopropanolic leaf extract of *Pimenta racemosa* on alpha amylase inhibitory assay**



#### 4. CONCLUSION

The current study was aimed to investigate *in vitro* antidiabetic activity of isopropanolic leaf extract of *Pimenta racemosa* by screening its possible phytochemicals may responsible for the actions. The phytochemical analysis confirms that the *Pimenta racemosa* leaf extract contain mixture of phytochemicals as reducing sugar, flavonoids, and alkaloids, etc. As

a result, we found that the isopropanolic leaf extract of *Pimenta racemosa* have inhibitory activity against  $\alpha$ -amylase and this therapeutic potentially could be exploited in the management of postprandial hyperglycemia in the treatment of Type 2 Diabetes mellitus. The results also indicated that the plant extract can be excellent options for biological and chemical analysis and can be further subjected for the isolation of therapeutically active compounds.

The antidiabetic effect of *Pimenta racemosa* also have been established by *in vitro* glucose uptake by yeast cells. The results indicate that *Pimenta racemosa* has potential as a crude drug and dietary health supplement for diabetic patients. Further studies are also required to elucidate whether the plant have antidiabetic potential by *in vivo* for corroborating the traditional claim of the plant.

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