Phytochemical Analysis And In Vitro Antidiabetic Activities Of Physalis Angulata Fruit Extracts

Sateesh Poojari*, Raju Porika* and Estari Mamidala**

*Research Scholar, **Assistant Professor, Department of Zoology, Kakatiya University, Warangal-506 009, Andhra Pradesh, India

Abstracts: **Introduction:** Diabetic mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrates, fat and protein. Plant based medicaments are extensively used for the treatment of various ailments of human beings. The main objective of the present investigation includes the preliminary screening of phytochemicals and evaluation of *in vitro* antidiabetic efficacy of *Physalis angulata* fruit extracts. **Materials and Methods:** Plant material was subjected to sequential extraction by maceration method by using different solvents. Primary screening of the fruit extract was conducted for detection of various types of phytochemicals, whereas antidiabetic activities of *Physalis angulata* were evaluated using inhibition of alpha amylase and alpha glucosidase enzymes **Results:** The study reveals the presence of phytochemicals such as alkalodis, saponins, phenols etc. Among the tested extracts of fruit methanol has been successfully inhibited both of the enzymes *in vitro*. The highest inhibition percentage 97.23 and 96.53 noticed against alpha amylase and alpha glucosidase at 100 µg/ml respectively. The results are comparable with the percentage 95.16% recorded with reference drug acarbose at 10 µg/ml. **Conclusion:** From the data obtained in the current studies, it was observed that the fruit extracts of *Physalis angulata* showed prominent antidiabetic properties *in vitro* and further the studies can be carried out for isolation of active principle responsible for activity. [Mamidala E et al NJIRM 2014; 5(2) :34-38]

Key Words: Antidiabetic efficacies, Acarbose, α- amylase, α- glucosidase.

Corresponding author: Dr. Estari Mamidala, Department of Zoology, Kakatiya University, Warangal-506 009, Andhra Pradesh, India., E mail: estari08@gmail.com

Introduction: Diabetic mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrates, fat and protein. The number of diabetes mellitus cases has been increasing worldwide on plant resources to generate the drugs that exhibit immense efficient and minimal side effect which help population of rural background India in the terms of availability affordable at low cost. Past 25 years, a drastic increase has been occurred in the utility of raw material of herbs in the system of alternative medicine[1].

Physalis angulata, belonging to family Solanaceae is a branched annual shrub and commonly referred to as camapu or balaozinh[2]. The infusions or extracts of this plant have been extensively used for the treatment of different types of diseases such as asthma, hepatitis, dermatitis malaria, and rheumatism[3-5]. It has been reported that A, B, D and F physalins and glycosides of this plant proved as significant anticancer agents[6-7,.5]. In context to above mentioned medicinal properties of this plant, the current investigation has been focused on *in vitro* determination of antidiabetic efficacies from fruit extract of *Physalis angulata*.

Material And Methods: Plant collection: The fruits were collected from the fields of Karimabad, District Warangal, Andhrapradesh, India. The authenticity of the plant was carried out by professor VS. Raju, Taxonomist, Department of Botany, Plant Systematic laboratory, Kakatiya University, Warangal.

Extraction procedure: The fruits were shade dried and grinded in homogenizer in to coarse powder. The 200 grams of powdered material was extracted by sequential maceration method using n-hexane, chloroform, ethyl acetate, acetone and methanol (non polar to polar). Concentration of extracts was carried out by rotavaporization at their boiling points and crude was collected and stored 4°C for further use.

Preliminary phytochemical analysis: All the solvent extracts of *Physalis angulata* fruits were tested for the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, phenolic compounds, using standard protocols [8-12].

Alkaloids: To detect the presence of alkaloids, a few drops of Mayer's reagent is added in solvent

free extracts. Alkaloids solution produces cream colored precipitate in presence of Mayer's reagent. Solvent free extract 50 mg is stirred with few ml of dilute HCl and filtered take few ml of filtrate and add 1 or 2 ml of Hager's reagent. A prominent yellow precipitate indicates the presence of Alkaloids.

Phenols: To test the Phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride ($FeCl_3$). Appearance of blue or green colour indicates presence of phenols. The extract (50 mg) is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Test for the carbohydrates: Reducing sugars two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars. Second, added to 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution. A red-brick precipitate showed the presence of reducing sugars.The extract (100 mg) is dissolved in 5 ml of water and filtered. To 0.5ml of filtrate, 0.5ml of Benedict's reagent is added. The mixture is heated on boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugars.

Cardiac Glycosides : To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride ($FeCl_3$) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites. 50 mg of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, to 2 ml of filtered 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia is added to it. Pink color indicates the presence of glycosides.

Tannins: To test the tannin phytochemical presence, in a test tube 1 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

Saponins: To test the saponin phytochemicals presence in various extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of foam indicates the presence of saponin.

***In vitro* enzyme inhibitory effects of *Physalis angulata* extracts.**

Alpha-amylase inhibitory activity: Screening of *Physalis angulata* for α-amylase inhibitors was carried out according to Xiao et al. (2006) with slight modification based on the starch-iodine test[13]. Alcoholic extracts of *Physalis angulata* of varied concentrations in 500 µL were added to 500 µL of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37°C for 10 min. Then 500 µL soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mM I2 and 5 mM KI). The colour change was noted and the absorbance was read at 620 nm on a microplate reader. The control reaction representing 100% enzyme activity did not contain any fruit extract. To eliminate the absorbance produced by fruit extract, appropriate extract controls without the enzyme were also included. Inhibition of enzyme activity was calculated as;

$$\text{Inhibition of enzyme activity (\%)} = A_c - A_s / A_c \times 100$$

Where as A_c=Absorbance of control

A_s=Absorbance of sample

α-glucosidase inhibitory activity: The effect of the fruit extracts on alpha glucosidase activity was determined according to the chromogenic method described by Kim et al., (2005)[14]. The substrate solution p-nitrophynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. Five units of alpha glucosidase (E.C. 3.2.1.20) were pre-incubated with 20 and 40 mg/ml of the different *Physalis angulata* extracts (n-hexane, chloroform,

ethyl acetate, acetone, methanol) for 15 minutes. Three millimolar (pNPG) as a substrate dissolved in 20 mM phosphate buffer, pH 6.9 was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 minutes and stopped by adding 2 ml of 0.1 M Na2CO3. The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from pNPG at 400 nm. The results were expressed as percentage of the blank control.

Inhibition of enzyme activity was calculated as;
Inhibition of enzyme activity (%)=A_c-A_s/A_c×100
Where as A_c=Absorbance of control
A_s=Absorbance of sample

Results: Preliminary phytochemical screening: All the fruit extracts of *Physalis angulata* were revealed to posses various types of major phytochemicals such as alkaloids, tannins, glycosides, phenolic compounds etc. The results were shown in the table 1.

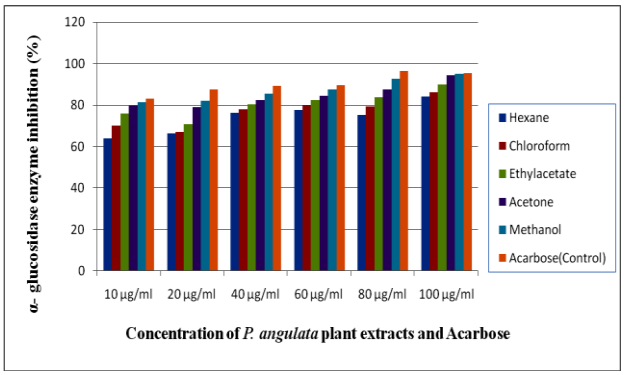
Inhibitory activities of *Physalis angulata* fruit extracts on α-amylase and α-glucosidase: Among the various *Physalis angulata* fruit extracts studied at various concentrations. Methanol extract was noticed significant percentage of inhibition of α-amylase and α-glucosidase enzyme activities when compared with that from other extracts (Figure 1 & 2). The highest inhibition percentage was recorded with the inhibition of α-amylase and α-glucosidase at 100 µg/ml are 97.23, 96.53 % respectively.

Table: 1. Preliminary phytochemical analysis from various *Physalis angulata* fruit extracts

Phyto chemicals	n-H	C	E	A	M
Alkaloids	+	+	+	+	+
Phenols	+	+	+	-	+
Tannins	+	+	-	+	+
Saponins	-	-	-	-	-
Glycosides	+	+	+	-	+
Carbohydrates	+	+	+	+	+

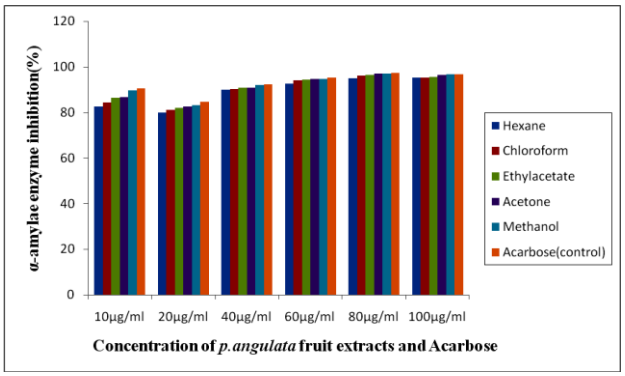
n-H- n-hexane, C- Chloroform, E-Ethyl acetate, A-Acetone, M-Methanol
+ indicates the presence of the compound
-indicates the absence of the compound

Graph: 1 Inhibitory effects of α- amylase by *Physalis angulata* fruit extracts at various concentrations



Next to methanol, hexane and chloroform extracts were also proved as significant inhibitors of the both enzymes with inhibition percentage 90.60, 84.71 and 83.18, 87.43% noticed for α-amylase and α-glucosidase enzymes respectively (Figure 1 & 2). Whereas, all other extracts are also exhibited considerable efficacy of inhibition at all tested concentrations. The inhibitory percentage observed for methanol extract is significantly comparable with reference standard drug acarbose 95.16% at10 µg/ml.

Graph 2 Inhibitory effects of α- glucosidase by *Physalis angulata* fruit extracts at various concentrations.



Discussion: The current investigation reports reveal that fruit extracts of *Physalis angulata* possessed numerous phytochemicals that are indirectly or directly attribute biological activity of extracts. However, all the extracts showed negative response for test carried out for the detection of saponin presence. Among the various extracts, acetone and ethyl acetate also showed negative response for the test carried out for the

identification of tannins and phenols and thus elucidates its poor or no activity biological activity. Whereas, methanol extract noticed the presence of all most all phytochemicals except saponin content. Thus, immense activity of methanol extract is might probably from the base of these phytochemicals.

In vitro determination of atidiabetic activity of the extracts tested revealed concentration dependent manner. The highest inhibition percentage was recorded with methanol extract compared with other extracts. Thus high polar solvents play vital role in the extraction of phytochemical in larger quantities, which exhibits direct relationship with its possessed activity.

By the enzyme inhibition assays, it has been clear that, mechanism of inhibition by fruit extract was not specific for both enzymes and share common reaction mechanism[15]. One of the strongest reasons that we believe is structural similarities of both enzymes is a key factor for non-specific manner of inhibition[15]. However, inhibition mechanism of these extracts may vary from standard acarbose, which delay or inhibit the mechanism of digestion i.e., final stage of carbohydrate metabolism conversion of disaccharide to monosaccharide and ultimately formation of glucose. However, this antidiabetic drug has been a problematic, as it is associated with gastro intestinal adverse effects, leading to accumulation of undigested starch in the large intestine (Madar 1989)[16].

The tendency to isolate the natural drugs from medicinally important plants has been drastically increased because of its high therapeutic value and non hazardous effects. Thus, naturally derived drugs have much more potency to replace the synthetic drugs that attribute adverse effects.

In the present study we have been hypothesized the antidiabetic efficacy of *Physalis angulata* fruit extracts were proved significant enzyme inhibitions *in vitro.*

Conclusion: The results obtained from the current study indicate that fruit extracts of *Physalis angulata* establish a good relationship with the antidiabetic properties and thus, the fruit extract of this plant can be directly used for the treatment of some forms of diabetic mellitus.

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