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Anti-diabetic activity of Physagulin-F isolated from *Physalis angulata* fruits

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Abstract

Type 2 diabetes is a chronic medical condition that requires regular monitoring and treatment throughout your life. Natural compounds from plant sources have been extensively used as traditional medicines from centuries. The present study was carried out to isolate and identify the putative antidiabetic compound from the Physalis angulata (PA) fruit. A compound, Physagulin-F was isolated from PA fruit extract. The isolated compound Physagulin-F (100, 300 and 500 mg/kg) of Physalis angulata fruit was undertaken to evaluate the anti-diabetic activity against streptozotocin (STZ)-induced diabetic rates. The compound 'Physagulin-F' produced significant ($p<0.5$) reduction in blood glucose level. The standard drug, glibenclamide (1.25 mg/kg) also produced significant ($p<0.05$) reduction in blood glucose level against STZ-induced diabetic rats. The results of this experimental study indicate that isolated compound 'Physagulin-F', possess anti-diabetic effects against STZ-induced diabetic rats.

Keywords: Physalis angulata, Physagulin-F, anti-diabetic, Type 2 diabetes

INTRODUCTION

Diabetic mellitus is a chronic metabolic disorder and poses a major challenge worldwide. According to Indian statistics, the current diabetic patients in India is around 40.9 million and it is expected to drastic rise up to 69.9 million by the end of 2025. Dramatically, India been emerged as diabetic capital of the world (Mohan et al., 2007; Joshi et al., 2007). Unless urgent preventive control steps are taken, it might become a biggest health problem in the running scenario. With reference to Indian Diabetes Federation (IDF) it has been estimated that every year 3.9 million deaths occurs due to diabetic mellitus and represents 6.8% of the total global mortality. In developing countries, anti-diabetic plants provide new oral anti-diabetic compounds that can counter part for high cost and side effect medicines. However, day by day the poor or non-availability of these medicines is the problematic for the people of rural back ground (Noor et al., 2008).

Plant derived drugs are commonly considered as less toxic and free from hazardous effects compared to synthetically derived drugs (Valiathan, 1998). Indigenous remedies have been extensively used in the treatment of various forms of diabetes mellitus since the time of Charaka and Sushruta (6th century BC) (Grover et al., 2001). According to the data obtained from World Health Organization (WHO) 21,000 plants which were massively used in the treatment of diabetes around the world, among these, 2500 species are off Indian origin. India is bestowed for larger producer of medicinal plants with a wide diversity of agro-climatic conditions is generally called as botanical garden of the world (Sultana et al, 2008). Number of pharmacological and clinical trials conducted using these medicinal plants have reported anti-diabetic effects by repair of β-pancreatic cells of islets of Langerhans (Ahmed et al., 2010). The present study was designed to evaluate the anti-diabetic activity of isolated compound Physagulin-F of the PA fruits against STZ-induced diabetic rats.

Physalis angulata, (a branched annual shrub) is commonly known as camapu or balaozinho in Brazil, belongs to Solanaceae (Januario *et al*., 2002). It is majorly distributed in tropical and subtropical regions of the world. The extracts or infusion of this plant is used in the treatment of a wide range of diseases such as asthma, hepatitis, malaria, dermatitis and rheumatism (Lin *et al*., 1992). Physalins (A, B, D and F) and glycosides such as Myricetin-3-Oneohesperidoside

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isolated from organic fractions of *Physalis angulata* reported significant anticancer activity on various tested cancer cell lines such as HA22T (hepatoma), HeLa (cervix uteri), lung adenocarcinoma, leukemia and epidermoid carcinoma (nasopharynx KB-16) cell lines (Islam et al, 2008). The biological properties of this plant includes anticancerous, antimycobacterial, anti-tumor, hypotensive, immunostimulant, anti-coagulant,etc (Januario et al.,2002). This Plant habitats in moist drained and sandy loamy soil and is renowned as effective stimulant for the immune system. The juice of this plant is used for the treatment of jaundice, earache, fever, bladder diseases etc. The fruit and aerial parts are extensively used in the treatment of constipation, sores, boils, cuts, intestinal and digestive problems (Sultana et al., 2008; Van Valkenburg and Bunyapraphatsara, 2002).

A literature survey of plants with anti-diabetic activity is an important prerequisite in the quest to quicken the search for novel DM treatments in India and beyond. Therefore, the current effort is a modest attempt to review the medicinal plant *Physalis angulata* with anti-diabetic activity. The use of herbal medicine is increasingly becoming more popular in many countries and this plant is used to treat DM in rural areas of Telangana State by traditional healers. Till to date the scientific research related anti-diabetic studies are not studied in this plant. Therefore, the present study was designed to evaluate the anti-diabetic activity of isolated compound Physagulin-F of the PA fruits against STZ-induced diabetic rats. The effect of Physagulin-F was compared to glibenclamide, which is often used as a standard drug.

MATERIALS AND METHODS

Plant material

The fully mature PA fruits were collected in August-September 2013 from the fields of Karimabad Village in Warangal District of Telangana State, India. The authenticity of the plant was carried out by professor VS. Raju, Taxonomist, Department of Botany, Plant Systematic laboratory, Kakatiya University, Warangal and voucher specimen was deposited in the Herbarium of the Metabolic Disorders Research Lab of the same University.

Preparation plant extract

The fruits were shade dried and grinded in homogenizer in to coarse powder. The powdered material was extracted by sequential maceration method using n-hexane, chloroform, ethyl acetate, acetone and methanol (non polar to polar) solvents. Concentration of extracts was carried out by rotavaporization at their boiling points and crude was collected and stored 4°C for further use. The weight of the residual extract was measured and percent yield was calculated.

Extract yield % = W1/W2 X 100;

Where, W1 = Net wt of powder in grams after extraction and W2 = total wt of powder in grams taken for extraction.

Preliminary phytochemical screening

All the solvent extracts of PA fruits were tested for the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, phenolic compounds, using standard protocols (Harbone, 1973; Rajendra Chary and Estari Mamidala, 2013; Rajendra Prasad and Estari Mamidala 2013a; Rajendra Prasad and Estari Mamidala 2013b).

Isolation and identification of the active compound

Thin layer chromatographic studies

Thin layer chromatography (TLC) profile with other physicochemical parameter can be good tool for standardization and validation of plants. TLC profile is simple and effective method for determination of the solvent system. TLC as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample on TLC volume 1µl by using capillary at distance of 1 cm at 1 track. Basic solvent system, hexane:ethyl acetate (100:0 to 0:100) were used in TLC to select the better solvent system to run column with methanol extract.

Column Chromatographic Studies

Column chromatography is a purification technique used to isolate compounds from a mixture. In column chromatography, the stationary phase is a solid adsorbent and the mobile phase is a solvent that is added to the top and flows down through the column. Separation is achieved based on the polar and non-polar interactions among the compounds, the solvent, and the solid stationary phase. Usually Silica or Alumina is used as the solid phase in order to setup the column. In this experiment, Silica was used as the solid medium for methanol extract. The column can be prepared using a column chromatography flask. Glass wool was inserted at the bottom of the flask to prevent the silica from escaping the column. The selected mobile phase (hexane:ethyl acetate-100:0 to 0:100) was continuously poured to the top with the aid of a dropper. The bottom outlet of the column was opened, allowing the eluent to flow through the column. As the eluent passed down the column, the compound fraction moved down the column. The separated fraction flowed out of the column where the different elutes were collected in separate test tubes. This was repeated until all the dissolved extract was adsorbed on to the silica gel. The collected elutes were tested in TLC up to single spot appeared.

Structure Elucidation

Based on TLC the elute is taken further ^{1}H NMR, ^{13}C NMR and Mass spectral studies for structural determination.

Animals

Adult albino rats of Wister strain (160–200 g), aged 8-14 weeks older were obtained from Animal House, University College of Pharmaceutical Sciences, Kakatiya University, Warangal and were used in the study. Rats were acclimatized for a period of 7 days before experimentation, housed in groups of six in polypropylene cages, lined soft wood shavings as bedding (renewed every 24 h), 12/12 h light/dark cycles, relative humidity 50-60% and at temperature 22±3°C, were fed with rat pellet diet (Gold Moher, Lipton India Ltd) and water *ad libitum* regularly. All the animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Care and Supervision on Experimental Animals (CPCSEA) and the study was approved by the Institutional Animal Ethics Committee (IAEC) of Kakatiya University.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the Physagulin-F were administered orally at the dose of 100, 300 and 500 mg/kg by intragastric tube and observed for the gross behavioral changes and mortality.

Anti-diabetic evaluation

Experimental induction of diabetes

Streptozotocin was purchased from Sisco Research laboratories Pvt. Ltd. Mumbai, India and was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 150 mg/kg body weight and injected intraperitoneally within 15 min of dissolution in a vehicle volume of 0.4 ml with 1 ml of tuberculin syringe fitted with 24 gauge needle, whereas normal control group was given citrate buffer only (0.4 ml). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of Streptozotocin.

Experimental protocol

Animals selected were fasted overnight and then divided into six groups (n=6) as follows:

Group I: Consisted of 6 rats which served as normal control and were given only distilled water daily.

Group II: Consisted of 6 Streptozotocin induced diabetic rats and served as diabetic control and were given distilled water only.

Group III: Consisted of 6 Streptozotocin induced diabetic rats and were treated orally with glibenclamide, used as a reference drug and was administered orally at 10 mg/kg as a suspension in 1% w/v CMC daily for 7 days, once in a day.

Group IV**:** Consisted of 6 Streptozotocin induced diabetic rats and were treated orally with isolated compound Physagulin-F at the dose of 100 mg/kg body weight daily for 7 days, once a day.

Group V: Consisted of 6 Streptozotocin induced diabetic rats and were treated orally with isolated compound Physagulin-F at the dose of 300 mg/kg body weight daily for 7 days, once a day.

Group VI: Consisted of 6 Streptozotocin induced diabetic rats and were treated orally with isolated compound Physagulin-F at the dose of 500 mg/kg body weight daily for 7 days, once a day.

Body weight and Glucose measurements

Body weight of all rats was measured during the course of study period. At the end of the experiment the blood (1ml) was collected by end tail vein cutting method and blood glucose level was determined by using one touch electronic glucometer using glucose strips (Vivek et al., 2007).

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± SD. Differences between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's *t*–test. p value < 0.05 were considered as significant.(Dixon and Jennrich, 1990).

RESULTS

The percentage of yield of extract macerated with various solvents are; Hexane (7.39 g) 3.695 %, Chloroform (6.22 g) 3.11%, Ethyl acetate (1.57g) 0.785 %, Acetone-(1.27 g) 0.65 % , Methanol (7.9 g) 3.95%. Methanol extract of *P. angulata* obtained the highest percentage yield to comparing to other solvent crude extracts (Table-1).

Table 1: Percentage yield of crude extract of four plants

Plant	Solvent extract	Weight in grams	% Yield
P. angulata (Fruit)	n-Hexane	7.39	3.695 %
	Chloroform	6.22	3.11 %
	Ethyl acetate	1.57	0.785 %
	Acetone	1.27	0.65 %
	Methanol	7.9	3.95 %

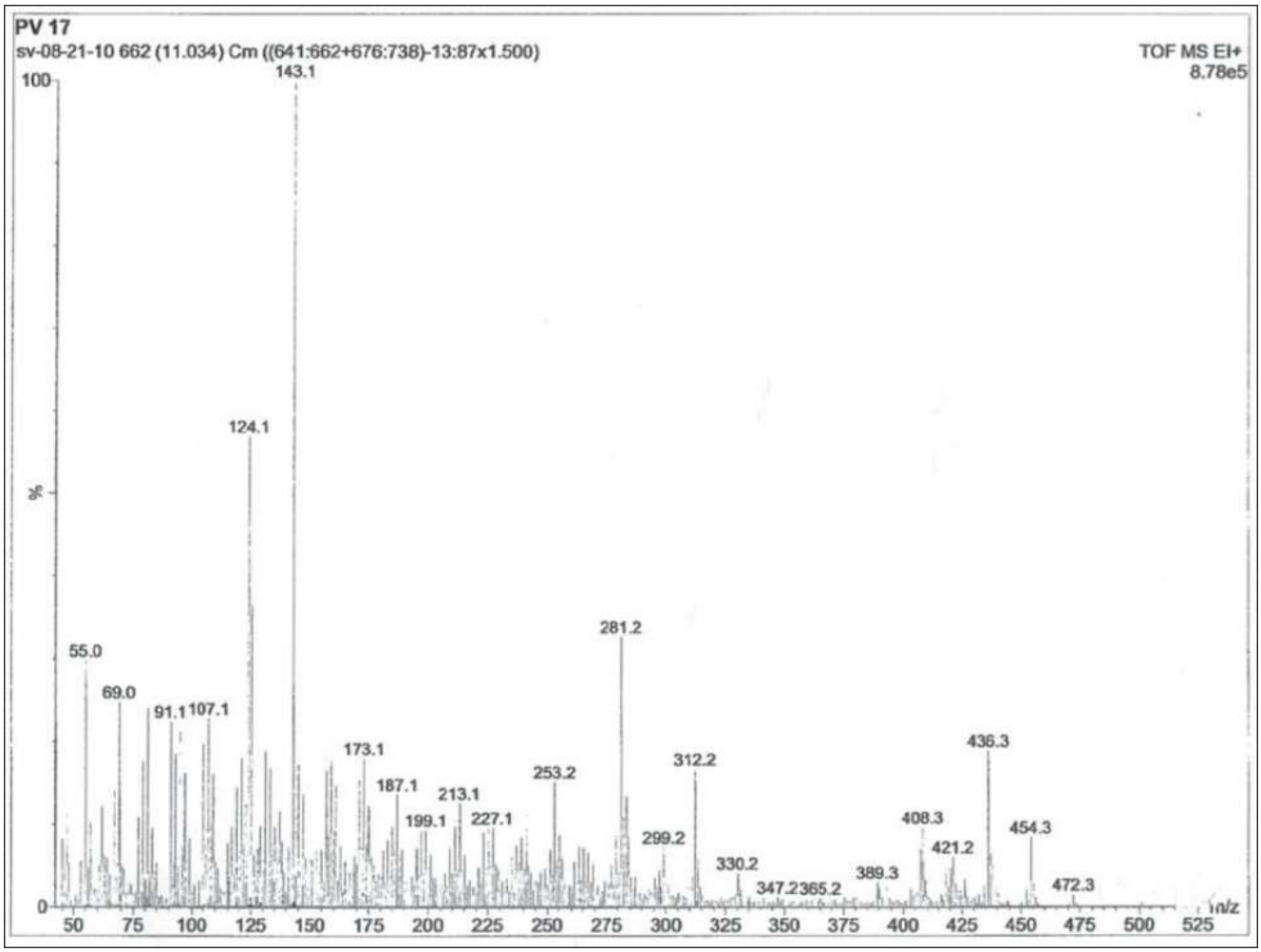
Isolation and Identification of the compound:

Methanol crude extract was filtered and concentrated under reduced pressure to yield a reddish brown residue. This crude extract was fractioned using silica gel (100-200 mesh) column chromatography and the components in crude extract eluted using solvents starting with hexane: ethyl acetate (100:0) and ending with hexane : ethyl acetate (0:100). 12 fractions were

Table-2. ^{1}HNMR and ^{13}C-NMR data of the Physagulin-F isolated from Fruit of *Physalis*

^{1}H NMR spetral data:
6.14dd (9.9,2.2), 6.69 ddd (9.9,5.1,2.2), 3.74 dt (22.2,2.2), 2.38 dd (22.2,5.1), 4.15 br s, 2.59 br dt (12.5,2.8) 2.38 br td (12.5,2.7), 2.51 br td (12.8, 2.8), 3.18 br td (12.8.2.6) 2.67 br td (13.0, 2.6), 1.42 m, 2.01 br t (13.0), 1.71 br d (12.8), 5.65 s,3.73 s,4.51 ddd (12.8,5.5,3.7), 2.33 br d (16.0), 2.13 br dd (16.0, 3.0), 1.91 s, 1.74 s,-OAc,2.25 s.
^{13}CNMR spectral data:
C-1(203.9),C-2(128.8) C-3(141.2) ,C-4(36.2),C-5(76.3),C-6(74.56),C-7(28.3),C-8(35.0),C-9(35.3),C-10(51.9),C-11(21.8), C12 (32.6), C13 (46.6), C14 (81.7), C15 (76.8),C16 (59.2), C17 (23.1), C18 (15.9), C19 (14.9), C20 (33.1), C21 (13.52), C22 (76.7), C23 (32.4), C24 (149.1), C25(121.9), C26 (160.3), C27(12.4), C28(20.5)-OAC

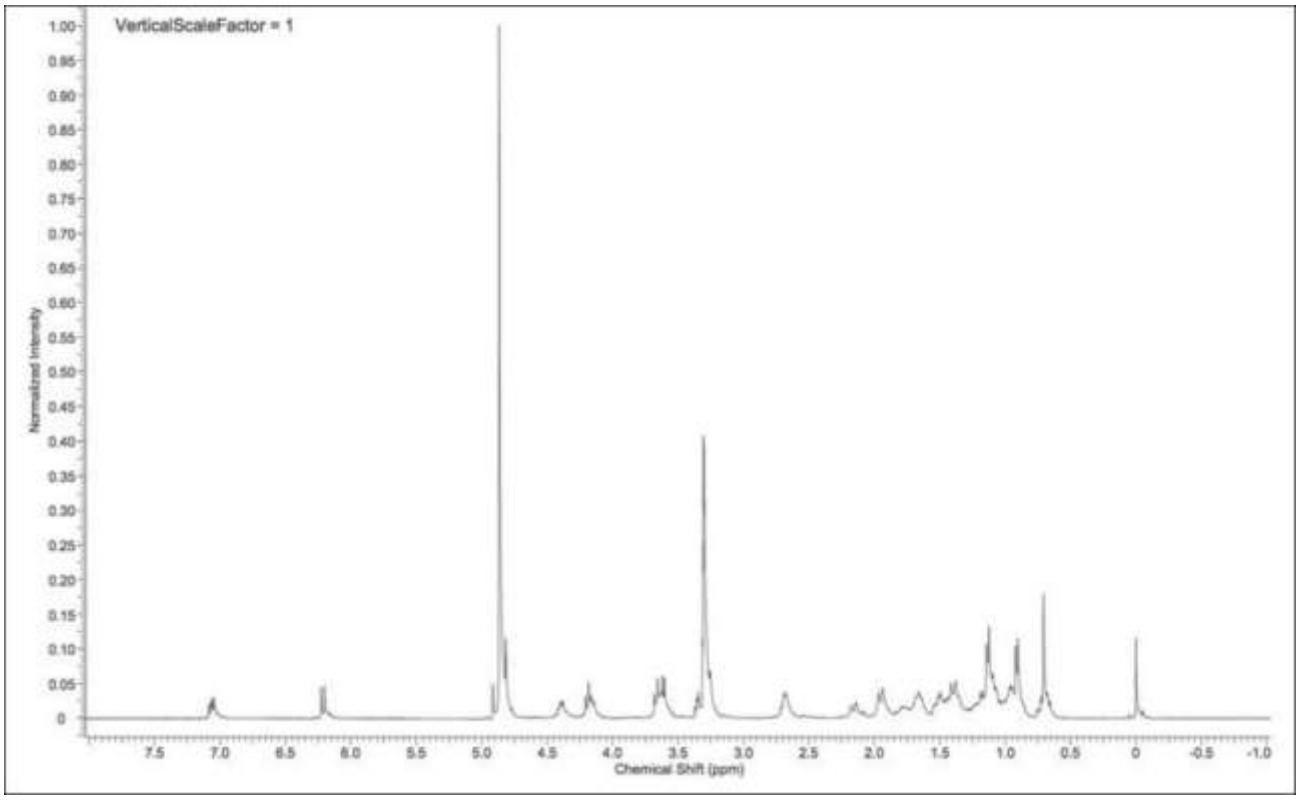
Figure-1. MS of Physagulin-F



yielded and designated as A1 to A 12. Among of which 1 fraction i.e. A4 which suggested possessing single compound in the crude extract. Fraction obtained from column was monitored according to the variations in composition indicated by the silica gel, 60, F254, TLC. The visualization of spots on the TLC plates was achieved by exposing TLC plates to iodine vapors after developing hexane and ethyl acetate as solvent system.

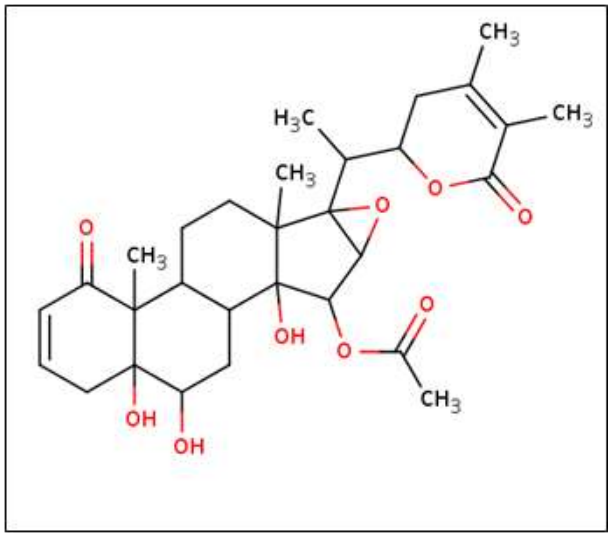
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Figure-2. ^{1}H NMR spectra of physagulin V. The spectrum was obtained at 400 MHz in MeOD at 27 °C with an acquisition time of 3.9715 s.



The TLC pattern of A4 strongly indicates as a single compound. In general, it can be understand that, even when an active compound locates with others in the mixture, it may not be able to perform its activity because of its presence in trace amounts it may be obscured by other substances present in the mixture. B4 fraction was designated as single compound and characterization was achieved through spectral analysis. It noticed greater antibacterial activity, and suggested that active compound is present in larger amounts.

Figure-3. Molecular structure Physagulin-F isolated form *Physalis angulate*



Based on Thin layer Chromatography fractionation and ^{1}H NMR spectral data the isolated compound is Physagulin-F belonging to the family of steroids and which possess the molecular weight $C_{30}H_{40}O_9$, average molecular weight is 544.6332 and IUPAC name is 6-[1-(4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl)ethyl]-2,16,17-trihydroxy-7,11 dimethyl-12-oxo-5-oxapentacyclo-octadec-13-en-3-ylacetate (Steroid Lactones). ^{1}HNMR, ^{13}CNMR data (Table-2) of the Physagulin-F isolated from fruit of *Physalis angulata* and molecular structure were shown in Figure-3.

Acute toxicity studies

This study showed no mortality up to the dose of 500 mg/kg body weight. So, the extracts safe for long term administration.

Anti-diabetic activity

At the end of the experiment (period of 7 days) animals of all groups expect group-III (diabetic control) showed considerable weight gain (Table-3). Group IV (Physagulin-F at 100 mg/kg) animals gained their body weight significantly of about 53.9%. Group V (Physagulin-F 300 mg/kg) animals notably gained their body weight by 61.7%. Group VI (Physagulin-F 500 mg/kg) animals gained their body weight by 73.5%. Whereas, animals of group II (Treated with 10 mg/kg glibenclamide) increasingly gained their body weight by 89.3% and animals of group III lost their body weight by 77.1%. Animals of group I (control) had normally gained their body weight by 34.8% (P<0.5). mg/kg glibenclamide significantly (P<0.05) higher blood glucose levels comparing with that from animals of group IV and V treated with 100 and 300 mg/kg Physagulin-F.

DISCUSSION

Diabetes has already been become a major lifestyle disease nowadays, which requires a proper management and control. However, the use of synthetic medicine is always not suggested due to its adverse effects. Thus, an alternate source for the sought out for the safer and affordable medicine, one can opt for medicinal plants which are comparatively possess lesser side effects. Due to the economic constraints, providing modern medical healthcare is still a far-reaching goal in developing countries, especially, in India. The most commonly used drugs such as aspirin, anti-malarial and anti-cancers etc. have been originated from plant sources. It is estimated that out of 250 000 higher plants (Vinatha Naini and Estari Mamidala, 2013), less than 1% of these have been screened pharmacologically and

Table-3. Percentage of Body Weight of Streptozotocin induced diabetic rats during the treatment with Physagulin-F isolated from *Physalis angulata* Fruit

Group	Before Treatment		After Treatment					
	Day-0		Day-1		Day- 4		Day-7	
	BW	GL	BW	GL	BW	GL	BW	GL
I (NC)	230.3 ± 1.0	98.16 ± 1.5	233 ± 0.7	102.8 ± 1.9	225 ± 0.1	100.3 ± 0.3	220 ± 0.13	101 ± 2.0
II (SD)#	232.3 ± 1.5	99.4 ± 0.5	231.3 ± 1.3	180.3 ± 0.8	225.6 ± 0.01	164.8 ± 0.6	230 ± 0.18	118.3 ± 1.5
III (DC)	234.6 ± 1.8	88.5 ± 1.3	230.6 ± 3.0	118.3 ± 1.7	224.3 ± 0.3	134.3 ± 0.8	200 ± 0.3	164.6 ± 0.21
IV (100*)	231 ± 1.2	100 ± 1.2	225 ± 1.4	172.3 ± 0.6	226.6 ± 1.2	152.3 ± 0.02	227.3 ± 0.7	140.6 ± 1.7
V (300*)	230.3 ± 0.01	101 ± 1.8	223.3 ± 0.3	164. ± 0.1	220 ± 1.6	145 ± 0.12	228.6 ± 0.12	133.6 ± 1.8
VI (500*)	227.5 ± 2.5	99.8 ± 1.1	224.5 ± 1.7	175 ± 0.2	225.5 ± 1.5	140.3 ± 0.19	225.9 ± 0.9	128.5 ± 0.6

Results expressed as SEM values (n=6), p<0.5
NC-Normal Control, DC-Diabetic Control, #SD-Standard (Glybenclamide 10 mg/kg), *Concentration of Fruit extract in mg/kg

As shown in Table-3 at the first week of treatment, the fasting blood glucose increased immediately after induction of diabetes with Streptozotocin in groups significantly higher (P<0.05). Animals in group IV, V and VI that were treated with Physagulin-F showed normal blood glucose levels compared with those animals in group II treated with standard drug. As from the 2nd day of treatment, there was a drastic reduction of blood glucose levels (P<0.05) were noticed from the diabetic animals of group VI. Animals of group II treated with 10 even very few in regard to diabetic mellitus. Therefore, it is very urgent to ascertain for naturally derive drugs from herbal medicinal plants for the treatment of diabetes.

The present work on antidiabetic activities of fruit methanol extract of *Physalis angulata* have been proven to be significant in both *in vitro* and *in vivo* experiment models. These study research on *in vivo and in vitro* antidiabetic activities of *Physalis angulata* fruit would become the first documentary which is being reported. These comparative studies of antidiabetic *in vitro* and *in*

vivo revealed that the methanol extracts of fruit exhibited a significant relationship with percentage of enzymes and glucose levels reduction with the concentration of extract (dose dependent manner). Methanol extract dominated methanol extract in all aspects of antidiabetic studies. The further investigation process must be carried out with methanol and methanol extracts for the isolation and characterization of active principle using bioassay guided fractionation.

Even though, this compound was previously reported for their isolation (Kazushi *et al*., 1992 and G. Krishna Et al, 2014), till today there are no scientific literature were available concerning to their biological activities, thus in continuity to isolation and structural elucidation the current studies are extended for determination of antidiabetic efficacy. Plant compounds continue to serve as viable source of naturally derived drugs for the world population are in extensive clinical use. Antioxidants are defined as substances that can delay or prevent the oxidation of lipids or other molecules by oxidizing chain reaction initiation or propagation and by many other mechanisms to prevent from disease (Zheng and Wang, 2001 and Rajendra Prasad Gujjeti and Estari Mamidala, 2014).

Plants with feasible antimicrobial activity should be assayed against appropriate microbial strains to confirm the possessed activity and to find out the parameters associated with it. The efficacies of these plant extracts on bacteria have been studied by numerous researchers present around world. Especially, relatively a large number of studies have been conducted on ethno-medicinal plants made of increased interest in isolation of large number of traditional natural products (Taylor, 1996). Plant-based medicaments have been become basis and alternative for many modern pharmaceuticals we use today. Ethno-pharmacological information based phytochemical research is generally considered as effective approach for the discovery of new anti-infective agents from higher plants. Knowledge related to the chemical constituents of plant is desirable, not only for the discovery of therapeutic agents, and also to economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants is also valuable for further discovering the actual value of folkloric remedies (Mojab *et al*., 2003 and D. Krishna Gopal Rao, 2013 and Sateesh Poojari et al, 2014). Medicinal plants have been provided a source of inspiration for novel drug molecules and made large contributions to human health. Their role is two-fold in the development of new drugs: (1) they have been become base for the development of a medicine (a natural blue print for the development of new drugs) or; (2) a phytomedicine to be used for treatment of various diseases.

Among the best examples of phytochemicals tannins have been found take important place in the prevention of several diseases. Theses form an irreversible complex with proline a rich protein resulting in the inhibition of cell protein synthesis. These protein reactions of tannins are known to play important in the treatment of inflamed or ulcerated tissues. Herbs that constitute tannins as their main components are astringent in nature and are utilized for the treatment of intestinal disorders such as diarrhea and dysentery. The biological properties of tannins have been reviewed and also reported that, tannins exhibit anticancer properties and can be used for cancer prevention. The other classes of phytochemicals that attribute medicinal properties are alkaloids. These alkaloids are also widely studied for their potential utility in the elimination and reduction of human cancer cell lines. Alkaloids are the largest groups of phytochemicals found in plants with amazing effects on human beings. Alkaloids have led to the development of powerful pain killer medications. Saponin inhibitory effects on inflamed cells have also been revealed. Steroidal extracts from some medicinal plants are also investigated and reported to exhibit antibacterial activities and antiviral properties. Flavonoids offer a wide range of biological properties such as antimicrobial, antioxidant properties, anti-diabetic, anti-inflammatory, anti-angionic, anti-allergic, cytostatic and analgesic (Igbinosa *et al*., 2009; Janovska *et al*., 2003; Devdatta Gopal Lad, 2014, Devdatta Gopal Lad, 2014, D. Krishna Gopal, 2013).

CONCLUSION

In the present study we have been hypothesized the antidiabetic efficacy of *P. angulata* fruit methanol extract in Streptozotocin induced diabetic rats. Our findings, directly indicate that the isolated compound Physagulin-F possess anti-diabetic activity resulted in high blood glucose level reduction compared with that from standard. This study supports the ethnobotanical usage of *P. angulata* in the treatment of diabetes and its associated complications. However, further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

Competing Interests Statement:

The authors declare that they have no competing financial interests.

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