

PHCOG MAG.: Research Article

Antihyperglycemic and antioxidant effects of *Talinum portulacifolium* leaf extracts in streptozotocin diabetic rats: A dose-dependent study

Ramesh Babu K., Vinay K., Sameena S.K., Prasad S.V., Swapna S., Appa Rao Ch.*

Department of Biochemistry, Sri Venkateswara University, Tirupati-517502. A.P, India

* Author for Correspondence: chippadar@yahoo.com

ABSTRACT

Hexane, ethanolic and aqueous extracts of *Talinum portulacifolium* leaves were prepared and given individually at different doses to normal and streptozotocin (STZ) induced diabetic rats after an overnight fast. The blood glucose levels were measured at 0,1,2,3,4,5 and 6 h after the treatment. The hexane extract at a dosage of 0.5g/kg b.w has shown maximal blood glucose lowering effect (64.7%) in diabetes rats. The same dosage did not produce any hypoglycemic activity in normal rats. The antihyperglycemic activity of hexane extract of *Talinum portulacifolium* leaves was significantly higher than that of glibenclamide, an oral hypoglycemic agent. All the extracts exhibited dose dependent scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, nitric oxide radicals and hydrogen peroxide. Further, all extracts had relatively lower reducing power, compared to that of ascorbic acid. The total phenolic content of hexane, ethanol and aqueous extracts were found to be 61, 100 and 114 mg/gm of the dry extract respectively. TLC of the above extracts using the DPPH as a spraying reagent revealed yellow spots against purple background indicating the presence of potent antioxidant compounds. In conclusion hexane extract of *Talinum portulacifolium* leaves possess both antihyperglycemic and antioxidant activities, while the ethanol and aqueous extracts possess only antioxidant activity.

KEYWORDS: Antihyperglycemic, DPPH radical, Reducing power, Streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease caused by an absolute or relative lack of insulin and/or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism. At least 171 million people worldwide have diabetes, this figure is likely to be more than double by 2030 and around 3.2 million deaths every year are attributable to complications of diabetes at the rate of six deaths every minute (1). Hyperglycemia is regarded as the primary cause of diabetic microvascular complications and contributing factor to diabetic macrovascular disease. Several major mechanisms

have been proposed for hyperglycemia induced-tissue damages, including increased polyol pathway, activation of protein kinase C (PKC) isoforms, hexosamine pathway, increased superoxide release from mitochondria and advanced glycation end products (AGE) formation (2–3). All these processes form reactive oxygen species (ROS) which alter intracellular redox state and provoke oxidative stress.

Studies indicate that hyperglycemia triggers the generation of free radicals and oxidative stress in capillary endothelial cells in the retina, mesangial cells in the renal glomerulus and neuron cells in the peripheral nerves (4). Therefore, it is essential to regenerate critical cellular

Antihyperglycemic and antioxidant effects of *Talinum portulacifolium* leaf extracts in streptozotocin diabetic rats

antioxidant responses to manage cellular redox status for preventing these diabetic complications resulting from hyperglycemia (5-6). Recent studies indicate a decrease in the occurrence of complications such as neuropathy and nephropathy in diabetic animal models with supplementation of different antioxidants such as vitamin E and lipoic acid. These results provide evidence that oxidative stress plays a role in the development of diabetic complications (7). Therefore, treatment strategies that focus on decreasing oxidative stress as well as enhancing antioxidant defense systems might present important options for treatment of diabetic complications. Hence compounds with both antihyperglycemic and antioxidative properties would be useful antidiabetic agents (8).

There is an increasing demand by patients to use the natural products with Antidiabetic activity, due to the side effects associated with the use of insulin and oral hypoglycemic agents (9), in addition they are not suitable for use during pregnancy (10). So that the management of diabetes without any side effects is still a challenge to the medical system. The ethnobotanical information reports about 800 plants that may possess antidiabetic potentiality. Folk medicine for diabetes from Rayalaseema reports 26 plants with antidiabetic activity. For a long time the tribal people of the Rayalaseema region in Andhra Pradesh, India have used the leaves of *Talinum portulacifolium* (Forssk: Portulacaceae) to keep away from Diabetes (11). The genus *Talinum* consists of approximately 500 species across the world. The family is cosmopolitan and it has 19 genera and more centered in South Africa and America (12). It is Perennial, suffrutescent, shrubby plant distributed from Rajasthan, India south wards in to the peninsular region; also found in Nepal. It is cultivated in Africa and, like spinach, is used as a vegetable. It is also said to be used as an aphrodisiac (13). The leaf powder of this plant mixed with boiled milk is used to treat diabetes (14). Nageswara Rao et al reported that the methanolic extract of *Talinum portulacifolium* leaves contain alpha-glucosidase inhibitory activity (15). But there are no systematic scientific studies on the antidiabetic and invitro antioxidant activities of different extracts of this plant (dose dependent study). The present study was undertaken to scientifically investigate the effect of different extracts of *Talinum portulacifolium* leaves on the blood glucose levels in streptozotocin (STZ) induced diabetic rats and invitro antioxidant activities.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Talinum portulacifolium* were collected from surrounding areas of Tirupati and identified by the

Botanist, Department of Botany, S.V. University, Tirupati. A voucher specimen (Herbarium Accession No.176) was deposited in the herbarium, Department of Botany, S.V. University, Tirupati. These leaves were shade dried and powdered; the powder was used for the preparation of different extracts.

Preparation of extracts

Hexane and ethanol extracts were prepared by successive solvent extraction in a soxhlet extractor; and extracts were then concentrated in vacuo to yield dense residues. To prepare aqueous extract the plant leaf powder (200 gms) was soaked in distilled water in a glass jar for 2 days at room temperature and the solvent was filtered. This was repeated three to four times until the filtrate gave no coloration. The filtrate was distilled and concentrated under reduced pressure in the Buchi rotavapour R-200 and finally freeze dried. The yield of the extracts were 6.7%, 8.3% and 21% for hexane, ethanol and water respectively. All the extracts were preserved in a refrigerator till further use. Preliminary phytochemical analysis was carried out in all 3 extracts by different methods of phytochemical analysis (16).

Induction of Diabetes

Diabetes was induced in male wistar albino rats aged 4 months (body weight 180–200 gms) by intraperitoneal administration of STZ (Sigma, Detroit, USA) (single dose of 50 mg/kg b.w) dissolved in freshly prepared 0.01M citrate buffer pH 4.5. After 48 hrs rats with marked hyperglycaemia (fasting blood glucose ≥ 250 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages, as per the guidelines of Institute Animal Ethics committee.

Experimental design

The animals were divided in to 9 groups and each group consisted of 6 rats.

- 1 Normal untreated rats
- 2 Diabetic untreated rats
- 3 Normal rats treated with 0.25 g/kg b.w of plant extract
- 4 Normal rats treated with 0.5 g/ kg b.w of plant extract
- 5 Normal rats treated with 0.75 g/ kg b.w of plant extract
- 6 Diabetic rats treated with 0.25 g/kg b.w of plant extract
- 7 Diabetic rats treated with 0.5g/kg b.w of plant extract

- 8 Diabetic rats treated with 0.75g/kg b.w of plant extract
- 9 Diabetic rats treated with 0.02g/kg b.w of glibenclamide

After an overnight fast the normal treated and diabetic treated rat groups received the ethanolic, aqueous extracts (dissolved in 1ml of distilled water) and the hexane extract (dissolved in 1ml of 5% Tween 80) by gastric intubation using a force feeding needle. Normal untreated and diabetic untreated rats were fed distilled water alone. But the control group for hexane extract received the vehicle (1ml of 5% Tween 80) instead of water. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5, & 6 hr after the administration of plant extract and blood glucose levels were determined by using dextrostix with Basic One Touch Accuchec Glucometer (Glucose oxidase method).

Antioxidant activity:

The following methods were used to evaluate antioxidant activity of the above 3 extracts.

DPPH radical scavenging activity

The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-colored methanol solution of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (17). 1ml of various concentrations of the extracts (25, 50, 75, 100 and 250 µg/ml) in methanol were added to 4ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517nm. The ability to scavenge DPPH radical was calculated by the following equation

DPPH radical scavenging activity (%) = $[(A \text{ control} - A \text{ sample}) / A \text{ blank}] \times 100$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound and A blank is only Methanol. Tests were carried out in triplicate.

Reducing power

The reducing power was determined according to the method of Oyaizu (18). Different concentrations of plant extracts (25, 50, 250 and 500 µg/ml) prepared in methanol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5ml, 1%). The mixture was incubated at 50°C for 20 min and 2.5ml of trichloroacetic acid (10%) was added to the mixture, which

was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard.

Determination of total phenolic compounds

Total phenols in plant extracts were determined by Folin-Ciocalteu reagent according to Singleton and Rossi (19) using Gallic acid as standard. 0.1ml (100 µg) of sample solution was made up to 3 ml with distilled water. About 0.5 ml of Folin-Ciocalteu reagent was added and mixed thoroughly and incubated for 3 min at room temperature. After incubation, 3ml of 20% Na_2CO_3 was added and mixed thoroughly and incubated in boiling water bath for 1min. The absorbance was measured at 650nm. The concentration of total phenols was expressed in terms of micrograms of Gallic acid equivalents.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green et al (20) and Marcocci et al (21). Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1ml of sodium nitroprusside (10mM), 1.5 ml of phosphate buffer saline (0.2M, pH7.4) was added to the different concentrations (25, 50, 75 and 100 µg/ml) of the plant extracts and incubated for 150 min at 25°C. After incubation 1ml of the reaction mixture was treated with 1ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthalenediaminedihydrochloride). The absorbance of the chromatophore was measured at 546nm. Butylated hydroxyl toluene (BHT) was used as a standard. The nitric oxide scavenging activity (%) was calculated by the following equation.

Nitric oxide scavenging activity (%) = $[(A \text{ control} - A \text{ sample}) / A \text{ blank}] \times 100$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound and A blank is only the phosphate buffer saline. Tests were carried out in triplicate.

H₂O₂ scavenging activity

The H_2O_2 scavenging ability of the plant extracts was determined according to the method of Ruch et al (22). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 10, 25, 50, 75 & 100 µg/ml concentrations of the plant extracts in 3.4 ml phosphate buffer were added to a H_2O_2 solution (0.6mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

Antihyperglycemic and antioxidant effects of *Talinum portulacifolium* leaf extracts in streptozotocin diabetic rats

The H_2O_2 scavenging activity (%) was calculated by using the following equation

$$H_2O_2 \text{ scavenging activity (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ blank}] \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Tests were carried out in triplicate.

TLC-DPPH antioxidant screening

This method is generally used for the screening of potential antioxidant activity in crude extracts. It involves the chromatographic separation of the crude plant extract, after which the developed chromatogram is sprayed with a colored radical solution and the presence of antioxidant compounds indicated by the disappearance of radical color. 10 μ l of each extracts was loaded as a 1cm band on the origin of TLC (Merck, Silica gel 60 F₂₅₄ plates). Plates were developed using the hexane: ethyl acetate (5:5), methanol (100%) and methanol: acetonitrile (7:3) for hexane, ethanol and aqueous extracts respectively. To detect antioxidant activity, chromatograms were sprayed with 0.2 % (w/v) DPPH in methanol (23). The presence of antioxidant compounds were detected as yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

Statistical analysis

All values are expressed as mean + S.D. The data were statistically analyzed by student's t-test.

RESULTS AND DISCUSSION

The effect of different doses of hexane, ethanolic and aqueous extracts of *Talinum portulacifolium* leaves on the fasting blood glucose levels of normal and diabetic rats are

given in Tables 1-3 respectively. The fasting blood glucose levels of diabetic untreated rats were significantly higher than those of normal untreated rats. The hexane extract at a dosage of 0.5 g/ kg b.w produced the maximum fall of 67.4% in the blood glucose levels of diabetic rats after 6 hrs of treatment (Table 1). The ethanolic and aqueous extracts also produced antihyperglycemic activity (a maximum of 13.8% and 26.8% respectively) at the dose of 0.5 g/kg b.w. in diabetic treated rats after 4th h of the treatment (Table 2–3). All extracts of *Talinum portulacifolium* leaves did not produce any hypoglycemic activity in normal treated rats. Treatment with Glibenclamide at a dosage of 0.02 g/kg b.w to the diabetic rats resulted in 30.9 % fall of blood glucose after 5 hrs of treatment.

Among all the doses of different extracts of TP leaves, the hexane extract at a dose of 0.5 g/kg b.w. produced maximum antihyperglycemic activity bringing down the blood glucose levels to almost normal. The efficacy of hexane extract (0.5 g/kg b.w.) in reducing blood glucose levels in diabetic rats is much higher than that of ethanol, aqueous extracts (0.5g/kg.b.w) and glibenclamide (0.02 g/kg b.w). The onset of antidiabetic action was observed from 1st hour after the treatment of the hexane extract and it was continued till the end of the 6th hour. But no hypoglycemic condition was observed in the treated diabetic rats during the treatment period. Drugs that normalize function, without causing hypoglycemia, would make attractive targets for diabetes (24). The antihyperglycemic effect of hexane extract was dose dependent upto 500 mg/kg b.w. However, the response decreased at the dose of 750 mg/kg b.w. Such a phenomenon of less antihyperglycemic response at higher dose is common with indigenous plants and has been observed with *Vinca rosea*, *Cinnamomum tamala*, *Aegle marmelose*, *Syzygium alternifolium*, *Momordica cymbalaria*, *Pterocarpus santalinus* (25-26). The decreased antihyperglycemic activity at doses higher than 0.5 g/kg b.w. could be due to reduced or

Table 1: Effect of hexane extract of *Talinum portulacifolium* leaves on fasting blood glucose levels (mg/dl) of Normal and Diabetic rats (Mean $\pm$ S.D).

Group	Blood Glucose at different hours after the treatment						
	0h	1h	2h	3h	4h	5h	6h
1	80 $\pm$ 5.2	86 $\pm$ 10.6	69 $\pm$ 14.9	81 $\pm$ 13.3	76 $\pm$ 14.9	71 $\pm$ 12.8	68 $\pm$ 10.2
2	287 $\pm$ 29.4 ^a	289 $\pm$ 32	292 $\pm$ 57	292 $\pm$ 63	289 $\pm$ 30.5	288 $\pm$ 47	287 $\pm$ 30.5
3	78 $\pm$ 1.41	83 $\pm$ 4.9	79 $\pm$ 4.2	78 $\pm$ 0.7	74 $\pm$ 2.8	76 $\pm$ 2.8	78 $\pm$ 2.8
4	75 $\pm$ 1.41	75 $\pm$ 8.48	85 $\pm$ 16.2	81 $\pm$ 14.1	73 $\pm$ 1.41	75 $\pm$ 5.6	81 $\pm$ 4.2
5	78 $\pm$ 2.8	83 $\pm$ 9.19	80 $\pm$ 5.6	81 $\pm$ 5.6	78 $\pm$ 2.1	78 $\pm$ 9.2	78 $\pm$ 2.8
6	281 $\pm$ 17.7 ^a	267 $\pm$ 19.5(5%)	250 $\pm$ 15.3(11%)	226 $\pm$ 15.1c(19.6%)	207 $\pm$ 19.3b(26.3%)	147 $\pm$ 13.6b(47.7%)	127 $\pm$ 8.3b(54.8%)
7	332 $\pm$ 55.6 ^a	307 $\pm$ 50.3(7.5%)	260 $\pm$ 21(21.7%)	247 $\pm$ 24.7(25.6%)	221 $\pm$ 8.5c(33.4%)	168 $\pm$ 11b(49.4%)	108 $\pm$ 5.5b(67.4%)
8	259 $\pm$ 38.4 ^a	254 $\pm$ 33	239 $\pm$ 35(7.7%)	223 $\pm$ 27.9(13.9%)	204 $\pm$ 24.8(21.2%)	182 $\pm$ 27.1(29.7%)	148 $\pm$ 20.4b(42.8%)
9	250.5 $\pm$ 9.3 ^a	227.6 $\pm$ 14(9.1%)	218.5 $\pm$ 13c(12.7%)	208.6 $\pm$ 14b(16.7%)	190.3 $\pm$ 14.7b(24%)	173 $\pm$ 13.6b(30.9%)	190 $\pm$ 12b(24.15%)

^ap<0.0001 compared with the initial level of blood glucose (0h) of normal rats.

^bp<0.0001 compared with the initial level of blood glucose of the rats (0h) in the respective group.

^cp<0.001 compared with the initial level of blood glucose of the rats (0h) in the respective group.

Table 2: Effect of ethanol extract of *Talinum portulacifolium* leaves on fasting blood glucose levels (mg/dl) of Normal and Diabetic rats (Mean $\pm$ S.D).

Group	Blood Glucose at different hours after the treatment						
	0h	1h	2h	3h	4h	5h	6h
1	79±6.9	91±6.5	87±9.9	81±8.7	79±10.3	76±4	83±8.3
2	279±29.4 ^a	278±35	281±66	283±79	280±35	276±27.5	278±30.5
3	78±1.41	77±6.3	76±9.9	80±1.4	83±2.8	79±2.8	80±2.1
4	74±11.9	78±0.4	81±12	88±6.3	75±10.6	92±0.7	72±5.6
5	76±0.7	77±0.4	78±7	79±2.8	76±4.2	74±2.8	79±0.7
6	278±14.7 ^a	307±23.5	276±29.1	265±24.1(4.6%)	252±20(9.3%)	291±50.3	308±39.3
7	289±7.3 ^a	345±11.5 ^b	300±7	274±11.1(5.1%)	249±15.5c(13.8%)	275±12.8(4.8%)	294±7
8	288±8.8 ^a	318±9 ^c	287±16.5	275±12(4.5%)	260±7.8c(9.7%)	310±26.6	330±32
9	250.5±9.3 ^a	227.6±14(9.1%)	218.5±13c(12.7%)	208.6±14b(16.7%)	190.3±14.7b(24%)	173±13.6b(30.9%)	190±12b(24.15%)

^ap<0.0001 compared with the initial level of blood glucose (0h) of normal rats.^bp<0.0001 compared with the initial level of blood glucose of the rats (0h) in the respective group.^cp<0.001 compared with the initial level of blood glucose of the rats (0h) in the respective group.**Table 3: Effect of aqueous extract of *Talinum portulacifolium* leaves on fasting blood glucose levels (mg/dl) of Normal and Diabetic rats (Mean $\pm$ S.D).**

Group	Blood Glucose at different hours after the treatment						
	0h	1h	2h	3h	4h	5h	6h
1	69±3.7	71±4.1	83±9.6	76±5.5	63±7	76±10	85±6.4
2	317±35 ^a	326±27.5	332±56	318±69	323±35	329±33.5	315±35.5
3	96±4.2	80±1.4	78±2.1	85±2.1	82±2.8	69±1.4	82±2.8
4	78±9.8	78±2.8	74±8.4	69±1.4	68±2.8	74±2.1	82±6.3
5	75±2.8	87±4.9	75±1.4	81±0.7	70±2.1	81±1.4	75±2.8
6	347±78 ^a	378±86.9	341±70.8	328±64.3(5.4%)	309±74.1(10.95%)	341±65.1(1.7%)	374±73.7
7	264±19 ^a	291±17.2	222±23.4	205±10.8 ^b (22.3%)	193±7.2 ^b (26.8%)	221±5.5 ^c (16.3%)	245±10.1
8	295±41.6 ^a	317±36.8	287±45.3	276±38.3(6.4%)	264±33.6(10.5%)	297±56.3	312±44.2
9	250.5±9.3 ^a	227.6±14(9.1%)	218.5±13 ^c (12.7%)	208.6±14 ^b (16.7%)	190.3±14.7 ^b (24%)	173±13.6 ^b (30.9%)	190±12 ^b (24.15%)

^ap<0.0001 compared with the initial level of blood glucose (0h) of normal rats.^bp<0.0001 compared with the initial level of blood glucose of the rats (0h) in the respective group.^cp<0.001 compared with the initial level of blood glucose of the rats (0h) in the respective group.

no effect of the components present in the extracts at higher doses (26). The phytochemical analysis revealed the presence of carbohydrates in ethanol and aqueous extracts. Due to that the blood glucose levels in treated rats were higher after 1h of treatment than FBG (0h) levels. The antihyperglycemic activity of *Talinum portulacifolium* may be due to its stimulating effect on the remnant beta cells or improvement in insulin action at cellular level or it could also be due to its insulin like effect.

Phytochemical analysis revealed the presence of steroids, triterpenoids, phenols, flavonoids and carbohydrates in hexane extract, phenols, and carbohydrates in ethanol extract, phenols, carbohydrates and saponins in aqueous extract. The antihyperglycemic and antioxidant activities of the extracts could be due to the presence of active principles phenols or flavonoids.

There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diabetes and other diseases (27). Many synthetic antioxidant

components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity (28). Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines etc) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (29). In the present study 6 different experiments were carried out to evaluate the antioxidant activity of extracts of TP leaves.

The antioxidants react with DPPH, a purple colored stable free radical, and convert it into a colorless α - α -diphenyl- β - picryl hydrazine. The amount of reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm. Substances that perform this above reaction can be considered as antioxidants and therefore radical scavengers (30). The DPPH radical scavenging activity was known to correlate well with the inhibitory capacity of lipid peroxidation of a test compound (31). As shown in Figure 1, all of these *Talinum portulacifolium* extracts showed appreciable free radical scavenging activities. The

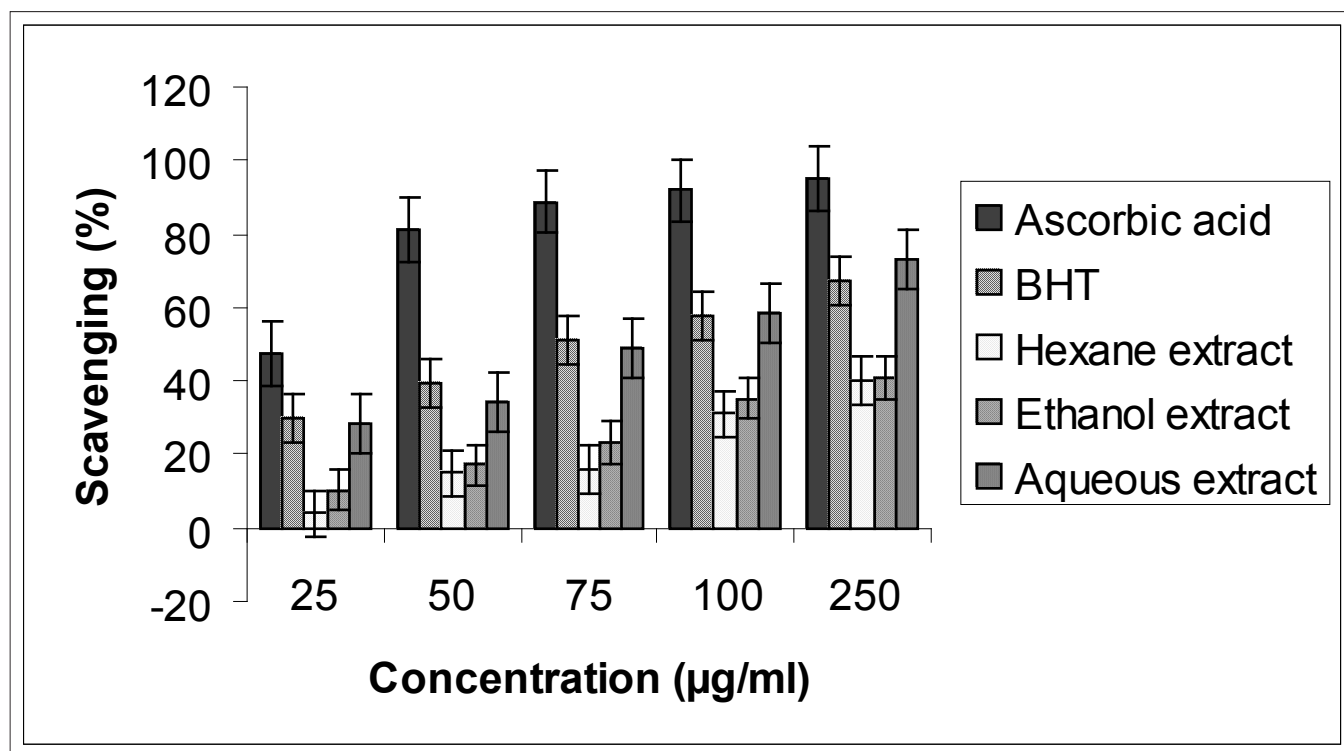


Figure 1. Scavenging effect of *Talinum portulacifolium* different extracts, standards BHT and ascorbic acid on 2, 2'-Diphenyl-1-picryl hydrazyl (DPPH) radical. Results are mean $\pm$ S.E of three parallel measurements.

best free radical (DPPH) scavenging activity was exerted by aqueous extract (73%) and it was greater than hexane (40%) and ethanol (41%) extracts. The standards BHT and ascorbic acid has shown 67% and 95% free radical (DPPH) scavenging activity respectively.

The reducing power has been used as one of the important antioxidant capability for medicinal herbs (32). In the reducing power assay, the presence of antioxidants in the all three extracts would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability. The reducing power of these *Talinum portulacifolium* extracts was dose-dependent (Figure 2). The highest activity exerted by aqueous extract followed by hexane and ethanolic extracts at a dose of 500 $\mu\text{g/ml}$. These extracts activities were compared with the ascorbic acid, which showed highest activity than that of all three extracts at the same dose.

The amounts of total phenolics varied widely in the different analyzed extracts and ranged from 61 to 114 mg/g of extract. This variation can be expected for plant extracts due to the presence of other constituents and/or the presence of different types of phenols. Among

plant extracts, the aqueous extract contained the highest amount of phenolics (114 mg/g) followed by ethanol (100 mg/g) and hexane (61mg/g) extracts. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables (33). But, in general, we have found correlation between antioxidant activity and total phenolic content.

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Overall, the aqueous extract of *Talinum portulacifolium* showed the highest nitric oxide scavenging ability compared to the hexane and ethanol extracts (Figure 3). At 100 $\mu\text{g/ml}$ concentration the aqueous extract showed highest nitric oxide scavenging activity than hexane (16%), ethanol (13%) extracts and standard BHT (14%). The nitric oxide scavenging abilities of above three extracts and standard BHT was shown below

Aqueous extract > Ethanolic extract > BHT > Hexane extract

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation

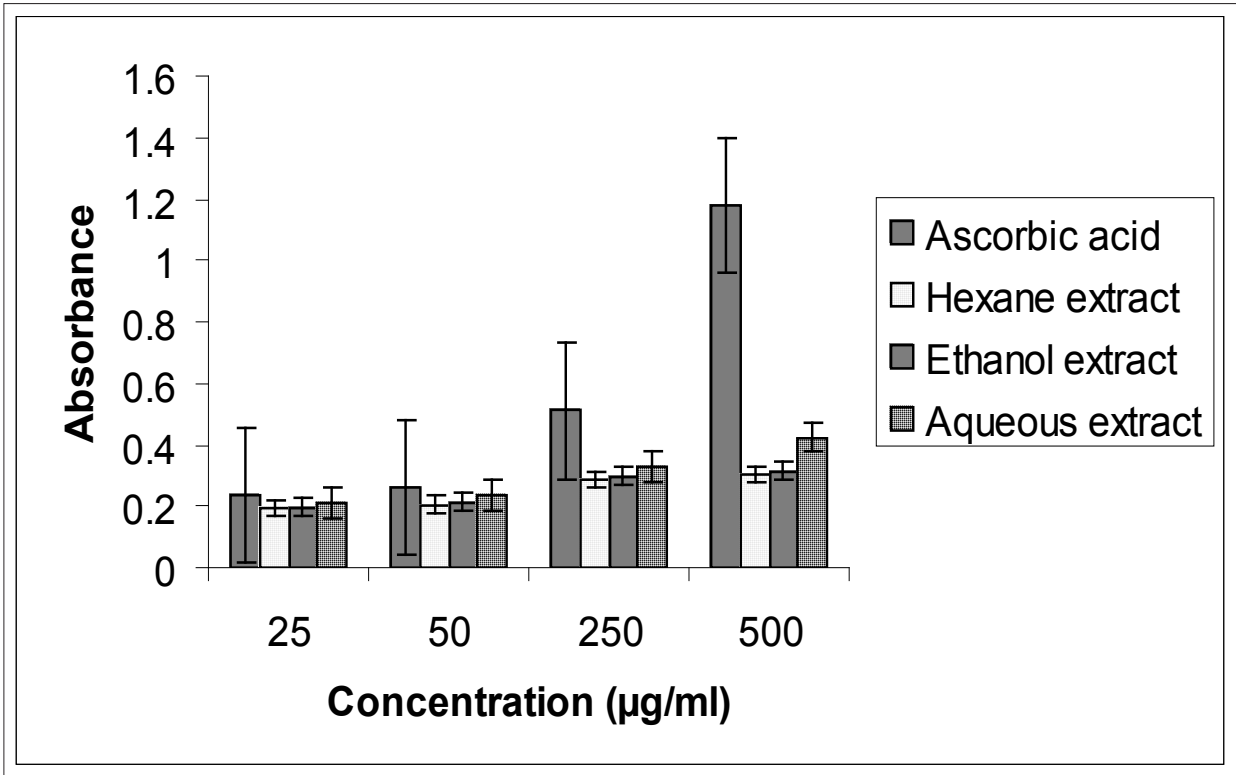


Figure 2. The reductive ability of *Talinum portulacifolium* different extracts and standard BHT. Results are mean $\pm$ S.E of three parallel measurements.

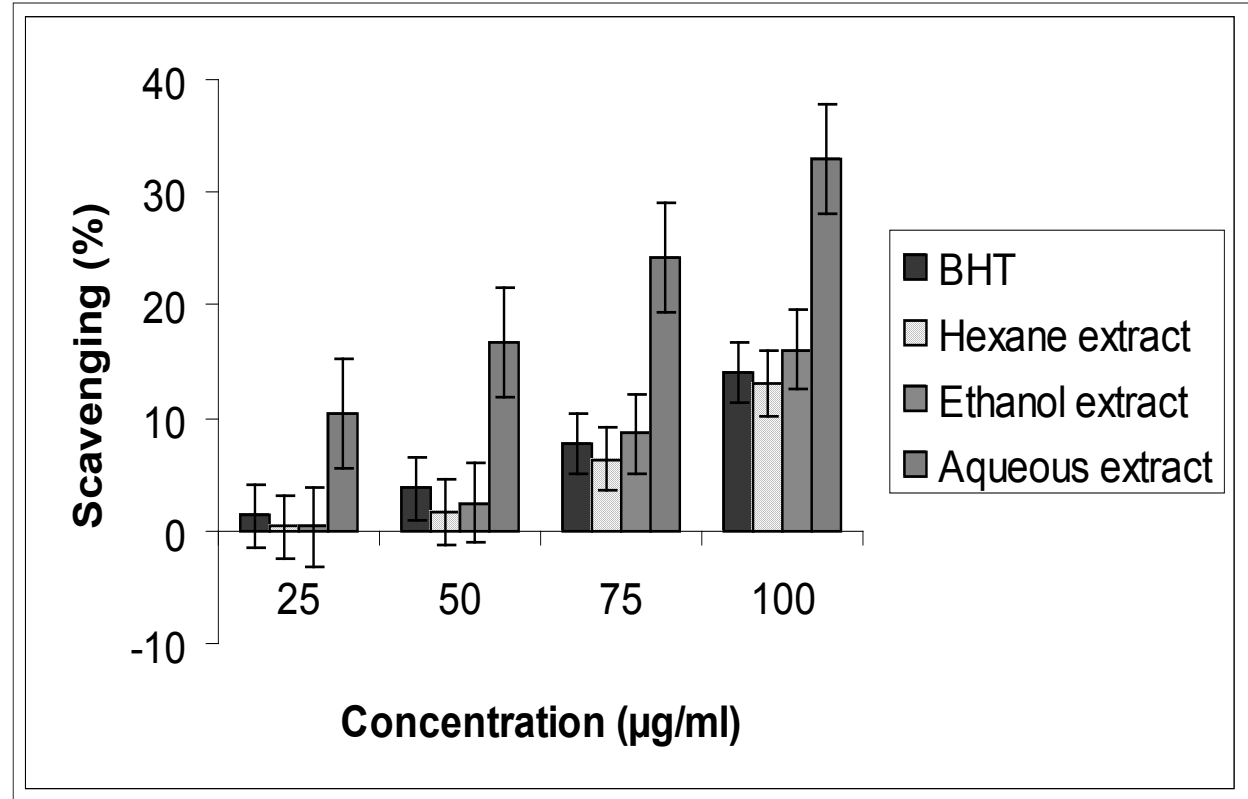


Figure 3. Scavenging effect of *Talinum portulacifolium* different extracts and standard BHT on nitric oxide radical. Results are mean $\pm$ S.E of three parallel measurements.

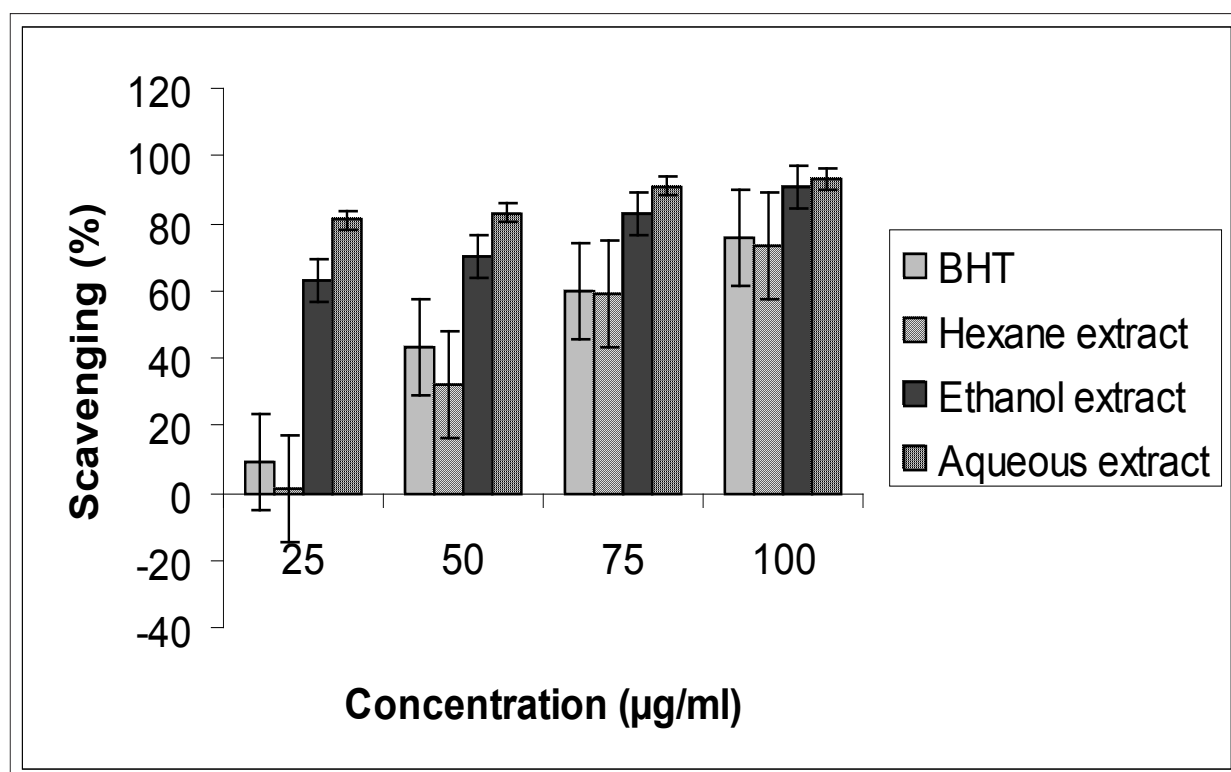


Figure 4. Scavenging effect of *Talinum portulacifolium* extracts and standard BHT on hydrogen peroxide. Results are mean $\pm$ S.E of three parallel measurements.

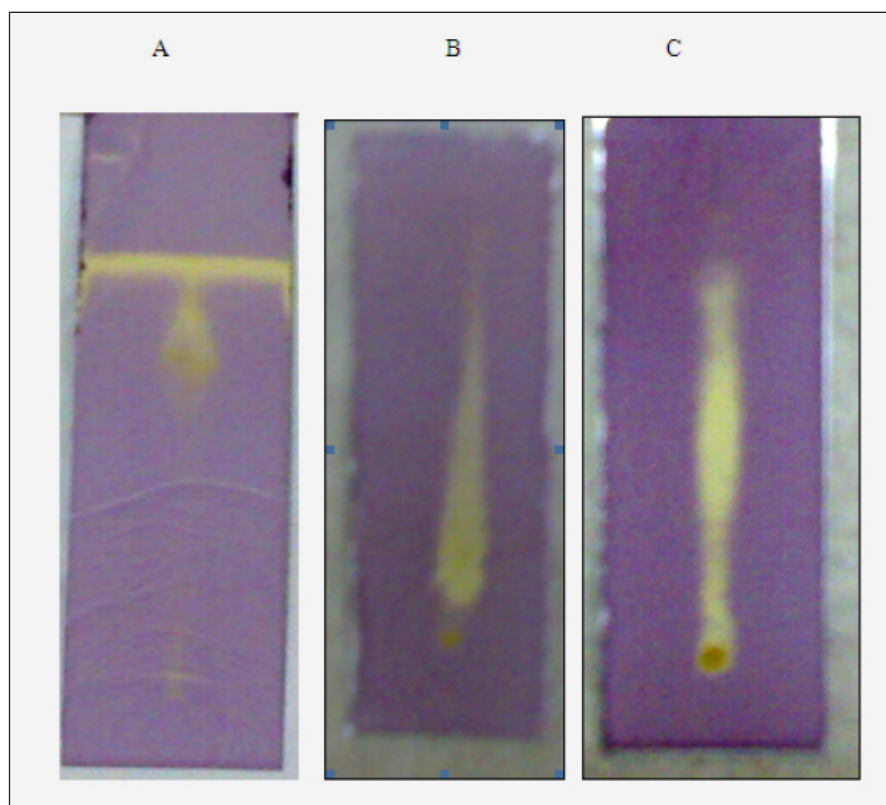


Figure 5. TLC-DPPH Chromatograms of hexane (A), ethanol (B) and aqueous(C) extracts.

of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} , and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (34). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Hydroxyl radical is the most reactive among reactive oxygen species (ROS) induces severe damage to adjacent biomolecules (35). The scavenging abilities of *Talinum portulacifolium* extracts on hydrogen peroxide were shown in Figure 4, each extract hydrogen peroxide scavenging activity was increased with increasing concentration of the extract (25-100ug). Aqueous extract (93%) had higher activity than hexane (73.5%) and ethanolic (91%) extracts. The standard BHT has shown 75.7% scavenging activity.

The TLC-DPPH chromatograms (Figure 5) reveals the presence of antioxidant compounds in all 3 extracts, which were detected as yellow spots against a purple background on TLC plate sprayed with 0.2% DPPH in methanol. From the chromatograms it was clear that aqueous extract having more potent antioxidant compounds than hexane and ethanolic extracts.

CONCLUSIONS

The present study showed that the hexane extract of *Talinum portulacifolium* not only possess antihyperglycemic activity but also antioxidant activity. This antioxidant property may reduce oxidative stress in diabetic patients which prevent diabetic complications. The antihyperglycemic activity of hexane extract of *Talinum portulacifolium* leaves may be due to its stimulating effect on the remnant beta cells or improvement in insulin action at cellular level or it could also be due to its insulin like effect. Further studies are under progress to identify the active antihyperglycemic compound(s) in hexane extract.

ACKNOWLEDGEMENTS

One of the authors K. Ramesh Babu gratefully acknowledge the University Grants Commission (UGC), India for providing financial assistance under the scheme of Research fellowship in sciences for Meritorious Students(RFSMS).

REFERENCES

- WHO (World Health Organization) Global Strategy on diet, physical activity and health www.who.int/dietphysicalactivity/publications/facts/diabetes/en; Accessed May, 2007
- Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. **414**: 813–820(2001).
- Evans J.L., Goldfine I.D., Maddux B.A., Grodsky G.M. Oxidative stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev*. **23**: 599–622(2002).
- Brownlee M. The pathobiology of diabetic complications. *Diabetes*. **54**: 1615–1625(2005).
- Heilig C.W., Concepcion L.A., Riser B.L., Freytag S.O., Zhu M., Cortes P. Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. *J Clin Invest*. **96**: 1802–1814(1995).
- Lee A.Y.W., Chung S.S.M. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J*. **13**: 23–30(1999).
- Rosen P., Nawroth P.P., King G., Moller W., Tritschlev H.J., Packer L. The role of oxidative stress in the onset and progression of diabetes and its complication. *Diabetes-metab res*. **17**: 189–212(2001).
- Baynes J.W. Reactive oxygen in the etiology and complications of diabetes. In: Ioanides C., Flat P.R.ed. *Drug diet and disease. Mechanistic approach to diabetes*. Hertfordshire: Ellis Horwood Limited. **2**: 203–231(1995).
- Holman R.R., Turner R.C., Oral agents and insulin in the treatment of NIDDM. In: Pickup J., Williams G. ed. *Text book of Diabetes*. Blackwell, Oxford; 467–469(1991).
- Larner J., *The pharmacological basis of therapeutics*, (MacMillan, New York, 1985) 7th ed.
- Nagaraju N., Rao K.N. Folk-medicine for diabetes from Rayalaseema of Andhra Pradesh. *Anc Sci Life*. **9**: 31–35(1989).
- Heywood V.H., *Flowering plants of the World* (Oxford University Press, U.K, 1978) 336.
- Anonymous, The wealth of India- A dictionary of Indian raw materials and industrial products, Vol **X**, (Council of Industrial and Scientific Research, New Delhi, 1974)113.
- Seetharamireddy T.V.V., Ramarao B.V.A., Prasanthi S., Herbal remedies for diabetes. In: Alikhan I. and Khanum A. ed. *Ethnomedicine and human welfare*. Ukkaz publications, Hyderabad. **3**: 44(2004).
- Nageswararao T., Kumarappan C., Thilagam E., Lakshmi S. M., Mandal S.C. Inhibition of Carbohydrate Digestive Enzymes by *Talinum Portulacifolium* (Forssk) Leaf extract. *J Complem Integr Med*. **5**(1): 2008.
- Harborne J.B., Baxter H., *Phytochemical Dictionary- A Handbook of Bioactive compounds from plants*, (Taylor and Amp Frost, London, 1983) **79**.
- Burits M., Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res*. **14**: 323–328(2000).
- Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Jap J Nutr*. **44**: 307–315(1986).
- Singleton V.L. and Rosi J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Vitic*. **16**(3). 144–158(1965).
- Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S. and Tannenbaum S.R. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem*. **126**: 131–138(1982).
- Marcocci L., Maguire J.J., Droy-Lefaix M.T. and Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract. *Biochem Biophys Res Commun*. **201**(2): 748–755(1994).
- Ruch R.J., Cheng S.J., Klaunig J.E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. **10**: 1003–1008(1989).
- Deby C., Margotteaux G. Relationship between essential fatty acids and tissue antioxidant levels in mice. *CR Seances Society Soc Biol Fil*. **165**: 2675–2681(1970).
- Porksen N. Therapy targeting β -cell survival and function in type 2 diabetes mellitus. *Diabetes Res Clin Pract*. **74**(2): S63–S69(2006).
- Kameswararao B. and Apparao Ch. Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* (Wt.) Walp. seed extracts in normal and diabetic rats. *Phytomedicine*. **8**(2): 88–93(2001).
- Kameswararao B., Giri R., Kesavulu M.M., Apparao Ch. Effect of oral administration of bark extracts of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. *J Ethnopharmacol*. **74**(1)69–74(2001).
- Rose W.M., Creighton M.O., Stewart D.H.P.J., Sanwal M., Trevithick G.R. In vivo effects of vitamin E on cataractogenesis in diabetic rats. *Can J Ophthalmol*. **17**: 61–66(1982).

Antihyperglycemic and antioxidant effects of *Talinum portulacifolium* leaf extracts in streptozotocin diabetic rats

28. Aruoma O.I., Cuppett S.L., Antioxidant methodology In vivo and In vitro concepts, (AOCS press, Champaign, Illinois, 1997) 41–172.
29. Formica J.V., Regelson W. Review of the biology of quercetin and related biflavonoids. *Food Chem Toxicol.* **33**: 1061–1080(1995).
30. Williams W.B., Cuvelier M., Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und-Technologie.* **28**: 25–30(1995).
31. Rekka E., Kourounakis P.N. Effect of hydroxyethyl rutosides and related compounds on lipid peroxidation and free radical scavenging activity. Some structural aspects. *J Pharm and Pharmacol.* **43**(7): 486–491(1991).
32. Duch P.D., Tu Y.Y., Yen G.C. Antioxidant activity of water extract of harng jyur (*Chrysanthemum morifolium* Ramat) *Lebensmittel-Wissenschaft und-Technologie.* **32**: 269–277(1999).
33. Luo X.D., Basile M.J., Kennelly E.J. Polyphenolic antioxidants from the fruits of *Chrysophyllum crinito* L. (star apple). *J of Agricultural and Food Chemistry.* **50**: 1379–1382(2002).
34. Halliwell B., Gutteridge J.M.C. Free radicals in Biology and Medicine. In: 2nd ed. *Free radicals, ageing and disease.* Clarendon press, Oxford; 279–315(1985).
35. Sakanaka S., Tachibana Y., Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (*kakinoha-cha*). *Food Chemistry.* **89**(4): 569–575(2005).