

In vitro Antidiabetic, Antioxidant and Antiglycation Activity of Ethanolic Leaf Extract of *Gomphrena globosa* (Linn.)

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ABSTRACT

G. globosa L. is an everlasting tropical annual plant reported to possess several biological activities for curing ailments such as arthritis, diabetes, asthma, bronchitis and also known to possess anti-tussive, anti-oxidant, anti-asthmatic and anti-cancer activities. The amino group of protein and carbonyl group of reducing sugar undergoes a non-enzymatic protein glycation reaction that leads to major complications in Diabetic patients. Several antiglycation agents of natural or synthetic origin are there to inhibit protein glycation reaction. But due to its toxicity, antiglycation agents of plant origin are gaining importance as an effective strategy to minimize diabetic complications. The aim of this study was to evaluate the antidiabetic, antioxidant and antiglycation activity of ethanolic leaf extract of *G. globosa*. The ethanolic leaf extract exerted its antidiabetic effect by inhibiting the activity of α -amylase ($p < 0.05$) resulting in the delayed digestion of the dietary carbohydrates and lowering the amount of glucose liberated. The highest scavenging was observed at 500 μ g concentrations and the percent inhibition was found to be 50.80% for DPPH assay and 57.24% ($p < 0.05$) for hydroxyl free radical scavenging assay. 25mM of glucose concentration showed the highest uptake of glucose. The glucose adsorption capacities of *G. globosa* ethanolic leaf extract was directly proportional to the glucose concentration in the medium resulting in significantly higher glucose adsorption. The effect of antiglycation activity of *G. globosa* ethanolic leaf extract was found to be increasing with increase in time. The inhibition of ethanolic leaf extract was found to be maximum (27.66%) at 72 hr, followed by 23.7% at 48 hr and 13.7% at 24 hr for fructation with ROS modification/UV/guanosine. This result indicates that ethanolic leaf extract of *G. globosa* possess significant activity at desirable concentration.

Key words: Antiglycation end product, *Gomphrena globosa*, Ethanol, Antiglycation, Antioxidant, Antidiabetic, Guanosine, ROS.

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INTRODUCTION

Type 2 Diabetes mellitus is one of the metabolic disorders arise due to insulin resistance and characterized by high blood glucose that it turns leads to major and minor complications. The use oral hypoglycemic agents and insulin have several side effects and hence the use

natural products with antidiabetic activity are rapidly increasing. Globe amaranth (*Gomphrena globosa*; family: Amaranthaceae; Tamil name: Vadamalli) an edible plant that consists of 120 species of white, red or purple head flowers. It is an annual dicot plant with a bushy appearance and grows 1-2 feet tall. The leaves of Globe amaranth are opposite, 4-6 in long, spread up to 1 ft and tolerate heat,^[1] drought and poor soil. Most commonly flower is magenta but also white, pale mauve varieties are also available.^[2,3] In Trinidad and Tobago, the plant is used to treat prostate problems and its flower reported to contain betacyanins that can be used as food colorants and antioxidants.^[4] It is effective in curing arthritis,

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diabetes, asthma, bronchitis and possesses antitussive, antioxidant, antiasthmatic,^[5] anticancer^[6] and analgesic activities.^[7] The crushed paste of this plant leaves is used for treating body sores.^[8] As type 2 Diabetes is alarmingly increasing worldwide, the major diabetic complication is due to hyperglycemia. This leads to the formation of advanced glycation end products (AGE) with increases in glucose availability.^[9] Protein glycation is a non-enzymatic maillard reaction that depends on amino groups of various aminoacids. During early stage glycation reaction, this amino group reacts with aldehyde or keto group in reducing sugars (glucose, fructose, xylose, galactose, xylose and deoxyribose) and form Schiff's base. The formed Schiff's base is rearranged into stable compounds called Amadori products. AGE are formed after the conversion of Amadori products into dicarbonyl compound and 3-deoxyglucosone that undergo further dehydration and rearrangement. In Diabetic patients, the increased blood glucose level will leads to increased formation of AGE that result in Diabetic complications^[9] and oxidative stress.^[10,11] The formed AGE will be recognized by receptors for AGEs in endothelial cells that results in the production of inflammatory response and oxidative stress through activation of nuclear factor κ B.^[10]

Methods such as spectrofluometry, mass spectrometry and SDS-PAGE analysis are used to estimate protein glycation. Inhibitors of type A, B, D and E that can inhibit the glycation reaction at different steps have been identified. The first designed drug to inhibit glycation was aminoguanidine^[9] but due to series of inhibitors side effects, new antiglycation agents of either synthetic or from medicinal plants with low toxicity, enhanced inhibition and antioxidant activity must be discovered^[11] to control and prevent Diabetic complications. Hence in this study *Gomphrena globosa* (L.) ethanolic leaf extracts was used to screen antidiabetic, antiglycation and antioxidant activities.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

G. globosa flowers were collected from Pudukkottai District (L.N. Puram region), Tamil Nadu, India, during the month of October to December, 2017 (Figure 1). The plant was identified by Dr. S. John Britto (Director, The Rapinet Herbarium and Center for Molecular Systematics, St. Joseph's College (campus), Tiruchirappalli-620002) and authenticated as *Gomphrena globosa* (L.). The Specimen No was CE 001 (Figure 2). The leaves of *G. globosa* was washed, dried, powdered and stored in air tight container. The ethanolic leaf

extract was obtained by maceration method and stored for further use.

Preliminary Phytochemical Analysis

G. globosa ethanolic leaf extract was subjected phytochemical analysis for detecting secondary metabolite as flavanoid, alkaloid, steroid, reducing sugar, tannins, gums and mucilages using standard procedure.^[12]



Figure 1: *Gomphrena globosa* (L.).

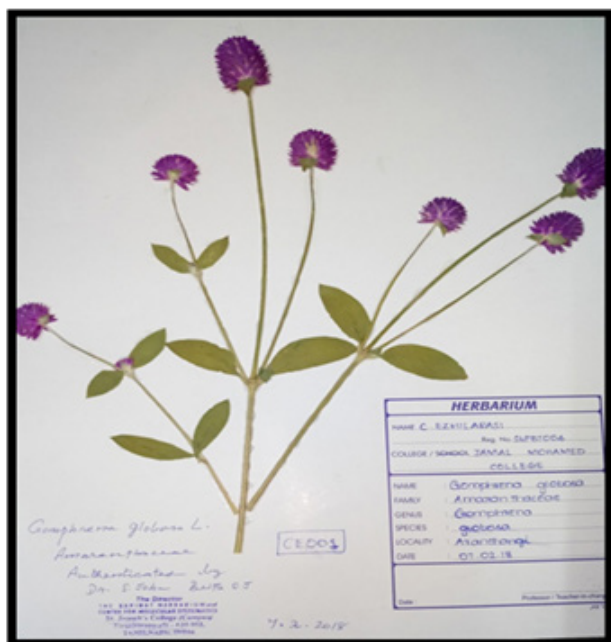


Figure 2: Specimen No: CE 001.

Evaluation of Antioxidant Activity of *G. globosa*

The antioxidant activity was determined by DPPH and hydroxyl free radical scavenging assays.^[13] The reaction mixture contains different concentration of leaf extracts (100 – 500µg) along with 2ml of DPPH. After 30mins of incubation at 37°C, the absorbance was measured at 517nm. The scavenging activity was measured by:

$$\% \text{ scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

Whereas A_0 is absorbance of blank and A_1 is absorbance of leaf extract.

The hydroxyl radical scavenging activity was measured by Deoxyribose assay. In brief, the reaction mixture containing ferric chloride, EDTA, 2-deoxyribose was mixed with different concentrations of ethanol leaf extracts and incubated at 1hr for 37°C. The reaction mixture was placed in water bath for 15 min at 95°C. To this 1ml of each TCA and TBA was added, centrifuged at 5000rpm for 15 min and OD was measured at 532nm.^[13]

In vitro Antidiabetic Effects of *G. globosa*

Alpha amylase inhibition assay

Various concentration of leaf extract (100 - 500µg) was mixed with alpha amylase solution and incubated at 25°C for 10 min. To this starch solution (1%) was added and again incubated. The reaction was terminated by adding DNS and absorbance was measured at 540nm.^[14] The inhibitory activity was measured as follows:

$$\% \text{ Inhibition} = \frac{A_b - A_c}{A_b} \times 100$$

Whereas A_b and A_c are absorbance of blank and leaf extract respectively.

Yeast cell assay

The ability of glucose transport across yeast cell membrane in presence of various concentration of leaf extracts was determined^[15] by using commercial baker's yeast. In brief, the reaction mixture containing 10% yeast suspension, various concentration of leaf extract (100 - 500µg), 1ml glucose solution (5, 10 and 25mM) was incubated at 37°C for 60 min. The glucose content was determined in supernatant after centrifugation. The percentage increase in glucose uptake was determined by,

$$\% \text{ increase in glucose uptake} = \frac{\text{OD test} - \text{OD blank}}{\text{OD test}} \times 100$$

Glucose adsorption capacity

25ml of 5, 10, 25 mM glucose solution was added to 1% of leaf extract and incubated at 37°C for 6hr. The

glucose content was measured in supernatant after centrifugation.^[16] The glucose bound concentration was calculated as:

$$\text{Glucose bound} = \frac{G_0 - G_6}{\text{Weight of the sample}} \times \text{volume of solution}$$

Whereas G_0 is initial glucose concentration and G_6 is 6hrs glucose concentration.

In vitro Glucose Diffusion Assay

The glucose diffusion assay of *G. globosa* was carried out by using a dialysis tube (7cm±15 mm). The tube contains 2mL of 0.22 mM D-glucose (dissolved in 0.15 M NaCl)^[17] and the dialysate glucose content was determined at 0, 1, 2 and 3 hr. GDRI percent was calculated by,

$$\text{GDRI \%} = 100 - \frac{\text{Glucose content (sample) (mg/dL)}}{\text{Glucose content (control) (mg/dL)}} \times 100$$

Antiglycation Activity of *G. globosa*

The reaction mixture containing Guanosine (100µg), leaf extract (100 µg), glucose and fructose (600 mg) was incubated for 24, 48 and 72 hr at 37°C. Guanosine alone served as a control. The absorbance was read at 254nm.^[16,17]

$$\% \text{ of Inhibition} = \text{OD Blank} \frac{(\text{OD sample} - \text{OD sample negative})}{(\text{OD blank})} \times 100$$

Antiglycation activity of ethanolic leaf extract under oxidative stress

Guanosine (100µg) along with leaf extract (100µg), glucose or fructose (600 mg) and hydrogen peroxide (100mM) was incubated for 24, 48 and 72 hr at 37°C and irradiated under 254nm UV light for 30 min. The absorbance was measured at 260nm.^[18,19]

In vitro antiglycation Activity

The reaction mixture containing glucose (25 and 5.5mM), BSA (15mg and 7.5mg/mL), with or without ethanolic leaf extracts of 100 - 500 µL (all dissolved in PBS pH 7.4) were prepared as per the Table 1 and Table 2. This was incubated for 5 weeks at 37°C and 50°C. The samples were drawn at 1st, 3rd and 5th week of incubation^[20]

Statistical Analysis

One way ANOVA followed by dunnet test (Control Vs test) was used to calculate the statistical difference. Microsoft Excel was used for DPPH and hydroxyl radical scavenging assay. The statistically significance values $P < 0.05^*$ and $p < 0.01^{**}$.

RESULTS

The preliminary phytochemical analysis revealed the presence of alkaloid, flavonoid, saponins, coumarins,

gum and mucilages Table 3. *In vitro* antioxidant activity of *Gomphrena globosa* leaf extract was analyzed by DPPH assay and Hydroxyl radical scavenging activity. The overall reducing power of the electron donating antioxidants in the reaction mixture was directly proportional to the change in absorbance. Highest Scavenging was observed at 500 μg concentrations and the percent inhibition was found to be 50.80%, where the standard showed 62.37% at 500 $\mu\text{g}/\text{ml}$ concentration (Figure 3). *G. globosa* ethanolic extract showed 57.24% scavenging activity when compared to standard that showed 67.93% at highest concentration (Figure 4).

The *G. globosa* extract showed appreciable 42.75% enzyme inhibitory activity against alpha amylase at 500 $\mu\text{g}/\text{ml}$ compared to the standard drug (54.64%) (Figure 5). The uptake of glucose by yeast cells in presence of extracts was presented in Figure 6 to Figure 8. The amount of glucose taken up by yeast cells was determined by measuring glucose remaining in the medium after a specific time which was found to be non-linear. The highest percent of uptake was seen in 25mM of glucose concentration, i.e. 55.11% at 500 $\mu\text{g}/\text{ml}$ (Standard showed 69.68%), followed by 42.69% at 500 $\mu\text{g}/\text{ml}$ (Standard showed 56.03%) in 10mM and 31.94% at 500 $\mu\text{g}/\text{ml}$ (Standard showed 49.48%) in 5mM concentrations.

G. globosa ethanolic leaf extract's glucose adsorption capacity was directly proportional to the glucose in the medium that results in significantly higher glucose adsorption. The maximum glucose adsorption was found to be at 25mM concentration with 16mMol/L of glucose bound, followed by 9.5mMol/L at 10mM in 1000 $\mu\text{g}/\text{ml}$ of leaf extract and 7.75mMol/L at 25mM glucose concentration in 500 $\mu\text{g}/\text{ml}$ of leaf extract (Figure 9).

Table 1: Concentration of Different Components used in Antiglycation Study.

S. No	Components of reaction	Concentration
1.	B: Buffer (PBS)	0.1M
2.	P ₁ : Protein (BSA) 1	15mg / mL
3.	P ₂ : Protein (BSA) 2	7.5mg/ mL
4.	G ₁ : Glucose 1	25.0 mM
5.	G ₂ : Glucose 2	5.5 mM
6.	PF ₁ : Plant Filtrate 1	100 μL
7.	PF ₂ : Plant Filtrate 2	200 μL
8.	PF ₃ : Plant Filtrate 3	300 μL
9.	PF ₄ : Plant Filtrate 4	400 μL
10.	PF ₅ : Plant Filtrate 5	500 μL

The diffusion of glucose in presence of *G. globosa* ethanolic leaf extract was time dependent and in dialysate more amount of glucose was found with

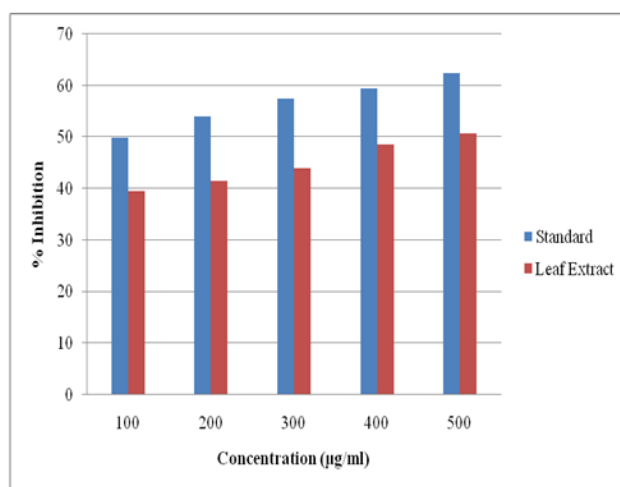


Figure 3: % Inhibition of DPPH free radical scavenging activity of *G. globosa*.

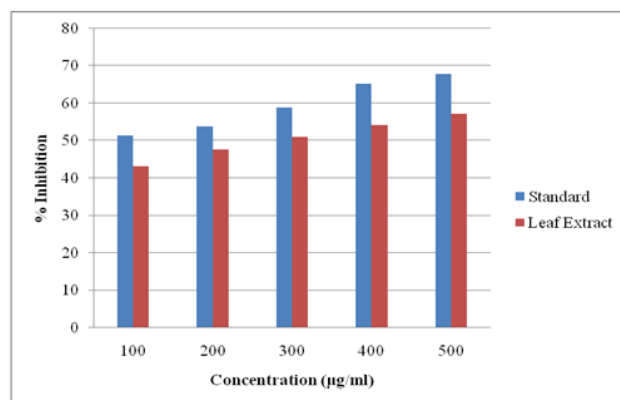


Figure 4: % Inhibition of ethanolic leaf extract of *G. globosa* for Hydroxyl free radical scavenging activity.

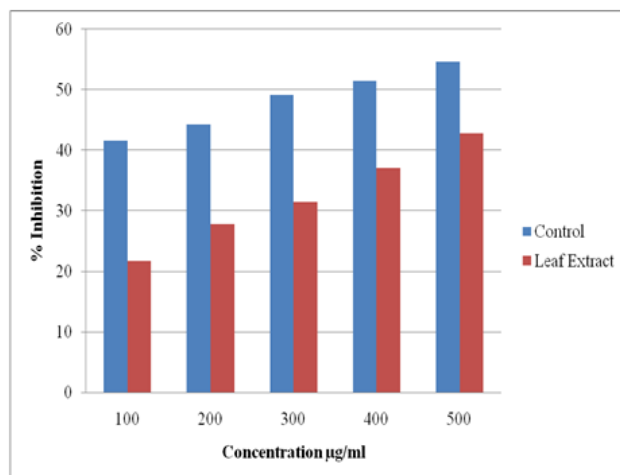


Figure 5: % inhibition of ethanolic leaf extract of *G. globosa* for α -amylase inhibitory assay.

increase in time from 0 to 180 min. The diffusion of glucose was inhibited in presence of leaf extract when compared to control. The concentration of glucose was estimated using standard curve (Figure 10). The GDRI % was found to be 11.12%, followed by 10.91% and 8.89% (Figure 11 and Figure 12). The concentration of

glucose content in dialysate was found to be decreasing as the time increases.

The absorbance value of glycated guanosine, fructated guanosine and reactive oxygen species of both at 260 nm for 24, 48 and 72 hr was represented in Figure 13. The effect of antiglycation activity of *G. globosa*

Table 2: Different Combination of Reaction used in Antiglycation Study.							
S.No	Reactions	S.No	Reactions	S.No	Reactions	S.No	Reactions
1.	$G_1 + P_1$	7.	$G_1 + P_2$	13.	$G_2 + P_1$	19.	$G_2 + P_2$
2.	$G_1 + P_1 + PF_1$	8.	$G_1 + P_2 + PF_1$	14.	$G_2 + P_1 + PF_1$	20.	$G_2 + P_2 + PF_1$
3.	$G_1 + P_1 + PF_2$	9.	$G_1 + P_2 + PF_2$	15.	$G_2 + P_1 + PF_2$	21.	$G_2 + P_2 + PF_2$
4.	$G_1 + P_1 + PF_3$	10.	$G_1 + P_2 + PF_3$	16.	$G_2 + P_1 + PF_3$	22.	$G_2 + P_2 + PF_3$
5.	$G_1 + P_1 + PF_4$	11.	$G_1 + P_2 + PF_4$	17.	$G_2 + P_1 + PF_4$	23.	$G_2 + P_2 + PF_4$
6.	$G_1 + P_1 + PF_5$	12.	$G_1 + P_2 + PF_5$	18.	$G_2 + P_1 + PF_5$	24.	$G_2 + P_2 + PF_5$

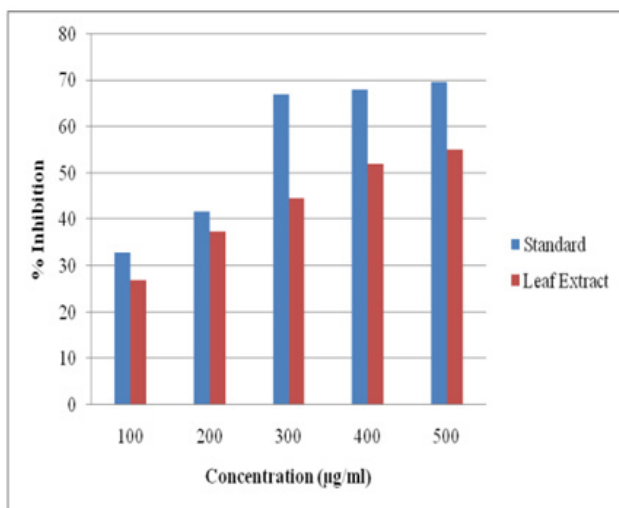


Figure 6: % inhibition of ethanolic leaf extract of *G. globosa* in 5mM glucose concentration.

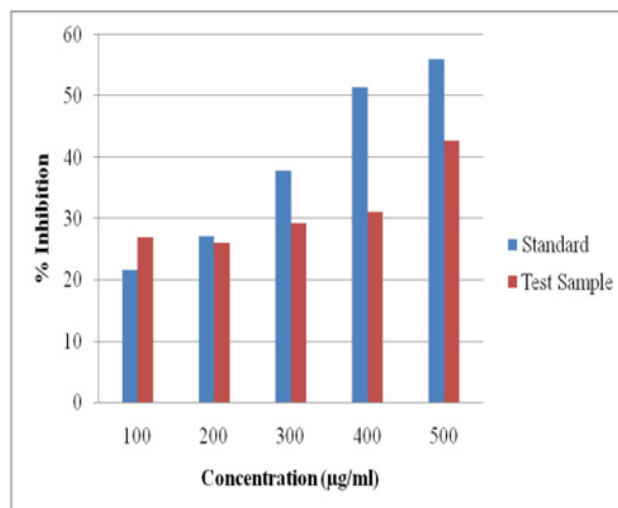


Figure 8: % inhibition of ethanolic leaf extract of *G. globosa* in 25mM glucose concentration.

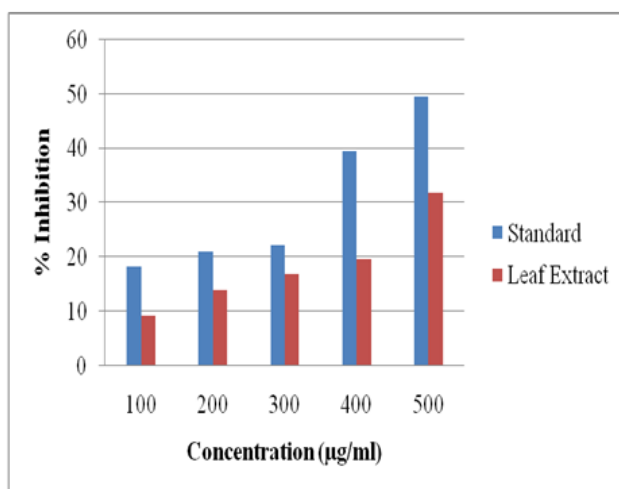


Figure 7: % inhibition of ethanolic leaf extract of *G. globosa* in 10mM glucose concentration.

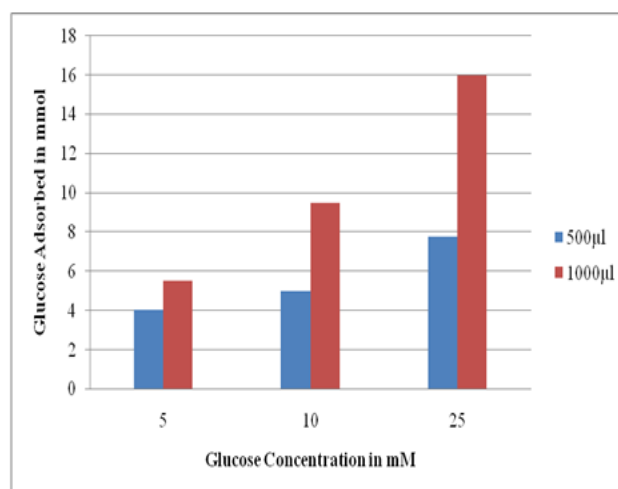


Figure 9: In vitro Glucose adsorption capacity of ethanolic leaf extract of *G. globosa*.

ethanolic leaf extract was found to be increasing with increase in time. The inhibition of ethanolic leaf extract was found to be maximum (27.66%) at 72 hr, followed by 23.7% at 48 hr and 13.7% at 24 hr for fructation with ROS modification/UV/guanosine. The inhibition of ethanolic leaf extract was found to be less for glycation with ROS modification/UV/guanosine. The antiglycation activity of ethanolic leaf extract with fructated guanosine showed 16.96% and glycated guanosine showed 15.8% at 72 hr (Figure 14 and Figure 15). The antiglycation activity of ethanolic leaf extract of *G. globosa* with different combination of glucose concentration, bovine serum albumin concentration and leaf extracts showed maximum absorbance of 0.198 for G2+P1+PF3, followed by 0.162 for G2+P2+PF4, 0.158 for G2+P2+PF3 and G2+P1+PF4 respectively (Figure 16 and Figure 17).

DISCUSSION

Plants are immensely potential and used as a folk medicine to cure several ailments due to its bioactive compounds such as alkaloid, flavanoid, terpenoids and phenolic compounds.^[21] *G. globosa* which is also called as

Bachelor's button contains various therapeutically active compounds and serves as a good candidate for assessing several biological activities. Phytochemical screening is most commonly used qualitative method for isolating and characterizing therapeutically valuable phytoconstituents present in plant sample. Maceration is a simple method to extract maximum bioactive compounds in less time, energy and solvent consumption.^[22] In this study the preliminary phytochemical analysis showed alkaloid, flavonoid, saponins, coumarins, reducing sugar, gum and mucilages.^[23] The major diabetic complications can be prevented by regulating blood glucose level in diabetic patients.^[21] Facilitated diffusion is involved in the glucose transport in Yeast cells.^[24] In this study, the yeast cells were treated with ethanolic leaf extract showed maximum glucose uptake in 25 mM and inhibited the diffusion of glucose in time dependent manner in glucose diffusion assay. The different combination of glucose, BSA and ethanolic leaf extract showed changes in protein glycation in time dependent manner at a concentration of 300 and 400 μ L. In earlier studies, the methanolic whole plant extract of *G. globosa* was reported to have anti-fungal and anti-bacterial

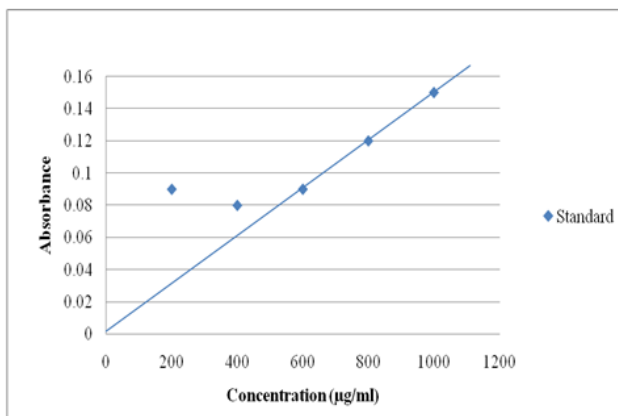


Figure 10: Standard Curve (glucose) for Inhibition of Glucose Diffusion Assay.

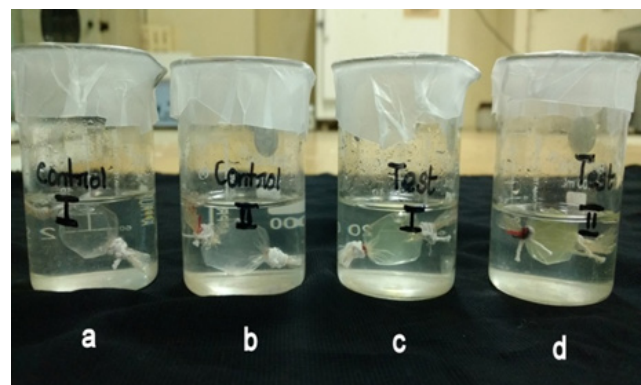


Figure 11: Inhibition of Glucose Diffusion Assay a, b - Control, c - leaf extract (500 μ g/ml), d - leaf extract (1000 μ g/ml).

Table 3: Phytochemical screening of ethanolic leaf extract of <i>G. globosa</i> (L.).		
S.No	Phytoconstituents	Results
1	Alkaloid	Positive
2	Flavonoids	Positive
3	Saponins	Positive
4	Gum and Mucilages	Positive
5	Coumarins	Positive
6	Reducing sugars	Positive

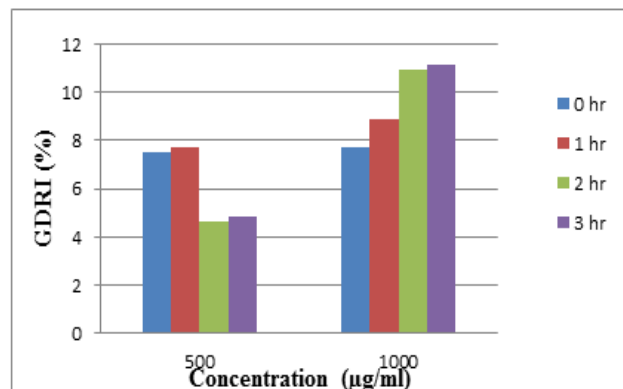


Figure 12: GDRI % of ethanol leaf extract of *G. globosa*.

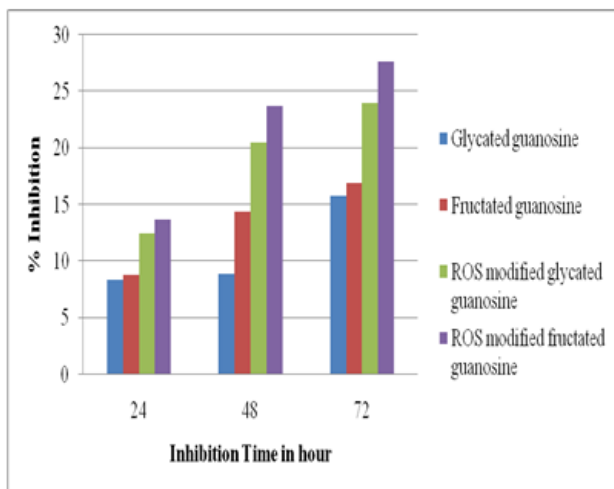


Figure 13: Inhibition percent of glycated, fructated and ROS modified glycated and fructated guanosine at 260 nm for various incubation times.

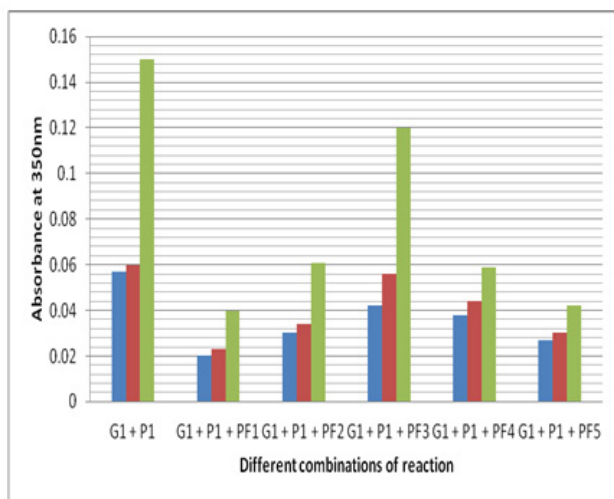


Figure 14: Antiglycation Activity of different combinations of reaction (G1+P1) by ethanolic leaf extract of *G. globosa*.

activity with significant antioxidant and cytotoxic activities.^[7] Heuer^[25] reported that *G. globosa* flower possess betacyanins such as gomphrenin I, II and III. *G. globosa* have been noted to use atmospheric sulfides for its growth.^[26] This plant also used as test plant for virus propagation and detection as it is susceptible to various plant viruses.^[27] Aqueous extract of *G. globosa* flower was also noted to be used as a substitute to synthetic indicators.^[28] It also reported that crude methanolic extract and ethanolic leaf extract of this plant also possess glucose lowering activity at a concentration of 400mg/kg of body weight^[29,30] in mice. Hence the above results indicates, ethanolic leaf extract of *G. globosa* possess antidiabetic and antiglycation activity that was proportionate to standard used in various assays.

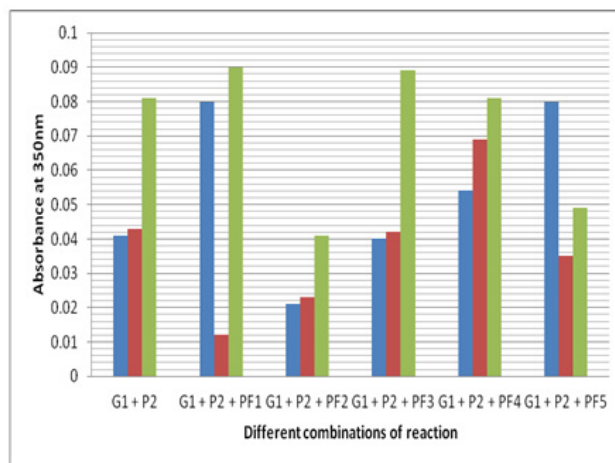


Figure 15: Antiglycation Activity of different combinations of reaction (G1+P2) by ethanolic leaf extract of *G. globosa*.

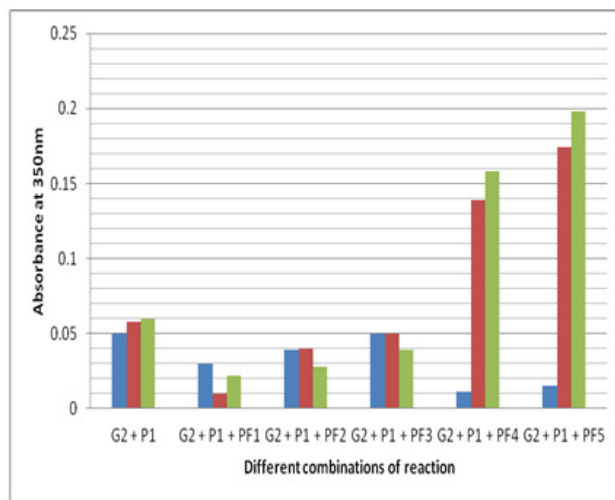


Figure 16: Antiglycation Activity of different combinations of reaction (G2+P1) by ethanolic leaf extract of *G. globosa*.

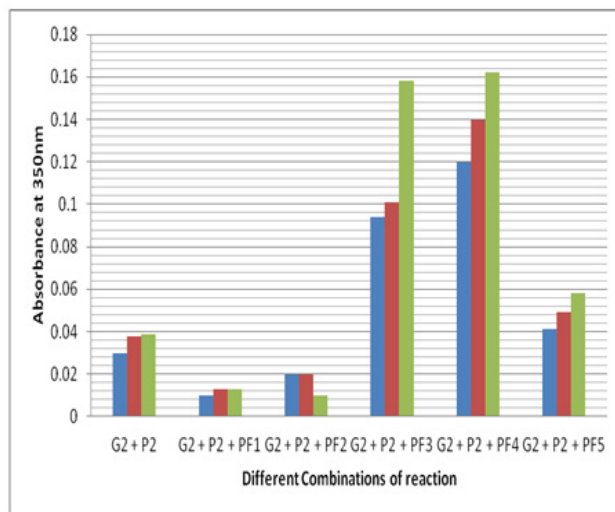


Figure 17: Antiglycation Activity of different combinations of reaction (G2+P2) by ethanolic leaf extract of *G. globosa*.

CONCLUSION

Plant secondary metabolites that act as phytoactive compounds are synthesized all over the plant parts. This phytoactive compounds work with nutrients and fibers that acts as a defense system against various diseases and stress conditions. This activity is possibly due to the presence of secondary metabolites and other constituents which showed hypoglycemic, antioxidant and antiglycation effects. Owing to the significance mentioned, this study was carried out to study the potential of ethanolic leaf extract of *G. globosa* towards antioxidant, antiglycation and antidiabetic effects under *in vitro* conditions. This revealed that this medicinal plant exert their antidiabetic effect through inhibition of α -amylase and glucose diffusion in dialysate resulting in the delayed digestion of the dietary carbohydrates and lowering the amount of liberated glucose with antiglycating and antioxidating properties. The isolation of individual phenolic compounds is necessary in this fraction and its effects on several *in vivo* studies are necessary to examine their role in drug development. Further, the lead compound from *G. globosa* can be used as a potent antidiabetic and anti glycation agent for treatment of diabetic complications.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

AGE: Advanced glycation end products; **SDS:** sodium dodecyl sulfate; **PAGE:** polyacrylamide gel electrophoresis; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDTA:** Ethylenediaminetetraacetic acid; **TCA:** Trichloroacetic acid; **TBA:** thiobarbituric acid; **DNS:** Dinitro salicylic acid; **OD:** optical density; **GDR:** glucose dialysis retardation index; **NaCl:** Sodium chloride; **mM:** millimolar; **BSA:** bovine serum albumin.

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