IN-VITRO ANTI-DIABETIC AND ANTIOXIDANT ACTIVITY OF *BOUGAINVILLEA SPECTABILIS* LEAVES

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ABSTRACT

Diabetes is a complex illness, as is well recognized. Diabetes (Type II) therapy is challenging because of the disease's underlying pathophysiological components. Diabetes has several consequences, including post-prandial hyperglycemia (PPHG). Glucosidase inhibitors, particularly alpha-amylase inhibitors, are a class of compounds that help manage PPHG. The goal of the current investigation was to ascertain the *in vitro* anti-diabetic and antioxidant activity of *Bougainvillea spectabilis* (Nyctanginaceae), which is a widely grown ornamental plant. From leaves, successive methanolic extracts were made. The *in vitro* antioxidant activity of a methanolic extract of *Bougainvillea spectabilis* leaves was determined by hydroxyl radical scavenging activity and ferrous-reducing antioxidant capacity assays. Plasma proteins and collagen are only two examples of the structural and functional proteins that can get glycated when blood glucose levels are elevated. Proteins may fragment, nitrous acid and lipids may be oxidized as a result of free radicals created from glycation. The present review shows that the methanolic extract of leaves of *Bougainvillea spectabilis* exhibited significant anti-diabetic and antioxidant activity, which is comparable to known standards.

Keywords: Multifactorial disease, inherent pathophysiological factors, post-prandial hyperglycemia, glycation, free radicals, fragmentation.

I. INTRODUCTION

Diabetes, sometimes referred to as diabetes mellitus (DM), is a complicated metabolic disorder defined by hyperglycemia, a physiologically abnormal state marked by persistently elevated blood glucose levels. Anomalies in the metabolism of carbs, lipids, and proteins are among the chronic and diverse signs of hyperglycemia. Anomalies in insulin activity or secretion are what lead to hyperglycemia. Diabetes can manifest itself in different ways and has a complex pathophysiology that leads to its progression. (1, 2).

As a result of hyperglycemia and the associated protein, lipid, and carbohydrate metabolic dysfunctions, a number of physiological organs are affected and unable to operate correctly. One of the most common complications of diabetes and one of the main contributors to morbidity and mortality associated with diabetes is atherosclerotic cardiovascular disease, which encompasses cerebrovascular disease, peripheral arterial disease, and coronary heart disease. (1, 3, 4)

A. Classification of Pathophysiology

The four main types of diabetes are type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and diabetes brought on by or linked to certain diseases, pathologies, or syndromes.

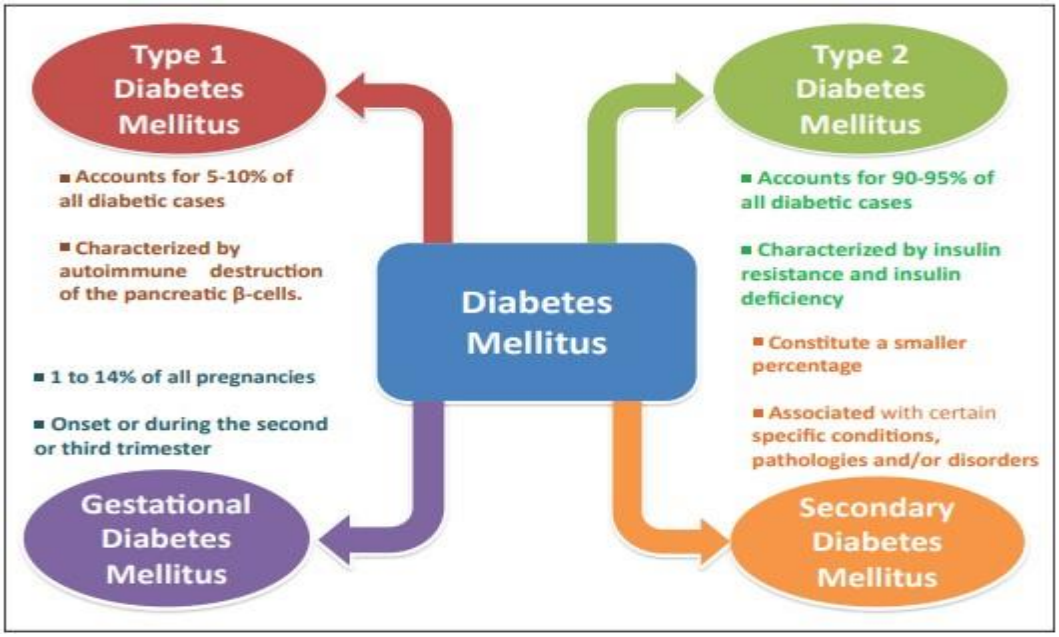

Figure 1: Types of diabetes mellitus

1. Type 1 diabetes mellitus

Insulin-dependent diabetes mellitus (IDDM), juvenile-onset diabetes, or type 1 diabetes are other names for type 1A diabetes mellitus (T1DM). It only accounts for 5–10% of all instances of diabetes and is brought on by an autoimmune disease that kills the beta cells in the pancreas, leaving the body without insulin and producing high blood sugar levels. Despite the fact that the precise etiology of this autoimmunity is not yet completely known, it is thought that both hereditary and environmental factors may be at play. Although it can sometimes develop slowly in adults (late onset), this autoimmune illness often manifests itself quickly in newborns and young children. (5, 6).

One immunological sign that sets T1DM apart from other autoimmune disorders is autoantibodies in particular. The specific immune-mediated cell death that characterizes the illness is linked to these autoantibodies. The autoantibodies include glutamic acid decarboxylase autoantibodies (GADAs) such as GAD65, islet cell autoantibodies (ICAs) to β-cell cytoplasmic proteins such as autoantibodies to islet cell antigen 512 (ICA512), autoantibodies to the tyrosine phosphatases, IA-2 and IA2α, insulin autoantibodies (IAAs), and autoantibodies to islet-specific zinc transporter isoform 8 (ZnT8). Among these autoantibodies, one might be used in clinical (1, 7).

2. Type 2 diabetes mellitus

90–95% of all instances of diabetes are caused by T2DM, formerly known as non-insulin-dependent diabetic mellitus (NIDDM) or adult-onset diabetes. There are two main anomalies of the insulin system that characterize this kind of diabetes:

- Insulin resistance
- Dysfunctional beta cells

Disruptions in numerous biological pathways that result in lower sensitivity of cells in peripheral organs including the liver, muscle, and adipose tissue to insulin are the cause of insulin resistance. To maintain normal blood sugar levels in the early stages of the illness, -cells overproduce insulin due to reduced insulin sensitivity. This condition is called hyperinsulinemia and helps prevent hyperglycemia. However, as time passes, -cell function begins to decline because the increased insulin release by these cells is unable to make up for the loss of insulin sensitivity. Eventually, -cell dysfunction leads to an insulin shortage, causing hyperglycemia. Although insulin levels are lower, the release of insulin usually prevents DKA (diabetic ketoacidosis) from occurring (8, 9, 10).

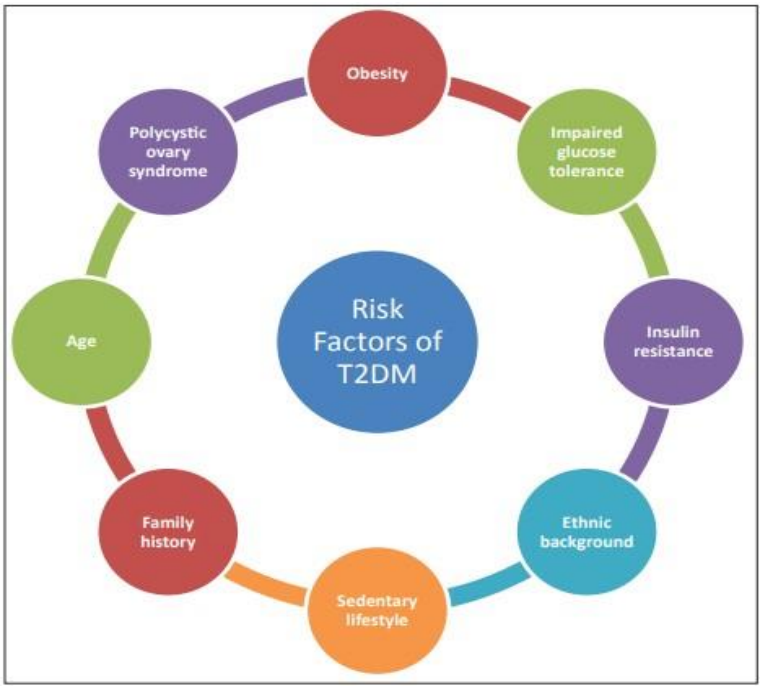

Figure 2: Risk factors of type 2 diabetes mellitus

3. Gestational diabetes mellitus (GDM)

GDM is the term for glucose intolerance or diabetes that is identified during pregnancy, typically in the second or third trimester. In the past, this also covered undiagnosed T2DM that might happen before or during pregnancy. The most recent recommendations from the International Association of Diabetes and Pregnancy Study Groups, however, only take into account GDM in high-risk women, such as obese women, with any degree of glucose intolerance. GDM differs from diabetes that was already present while a woman was not pregnant and usually goes away after delivering or ending the pregnancy. Fasting and post-meal blood glucose levels are often lower than normal during the early stages of pregnancy. However, during the third trimester, blood glucose levels increase, and if they approach diabetic levels, it is called gestational diabetes mellitus (GDM) (1, 11).

4. Other types of diabetes

In addition to T1DM, T2DM, and GDM, diabetes has been associated with various other illnesses and disorders. However, these types of diabetes are less common compared to the overall incidence of diabetes. Endocrinopathies, exocrine pancreatic disorders, diabetes arising from genetic anomalies in insulin action, diabetes caused by monogenic deficits in -cell activity, and those are some of the various kinds of diabetes. Numerous drugs are utilized for diabetes treatment, such as insulin and various oral hypoglycemic agents like sulfonyl, metformin, glucosidase inhibitors, and froglitazone. It is crucial to note that these drugs have been associated with numerous adverse effects, including liver problems, lactic acidosis, and diarrhea (12). The search for the development of more powerful natural antidiabetic medicines has been studied due to the side effects of conventional treatment. Traditional herbal medicine, one of the oldest cures known to mankind, has recently received a lot of attention.10 numerous traditional herbs may also safeguard the organs involved in diabetes mellitus due to their hypoglycemic and antioxidant effects (13).

The physiological and biochemical abnormalities of the diabetic condition, which are frequently linked to alterations in the lipid, protein, and carbohydrate metabolisms, have been extensively studied in vitro. Through a variety of processes, including metabolic inefficiency and changes in the structure and characteristics of proteins, lipoproteins, and DNA, tissue damage is caused by oxidative stress with an increase in reactive oxygen species brought on by insulin resistance linked with decreased insulin production (17, 18).

A woody, thorny vine known as *Bougainvillea spectabilis* is grown in tropical and subtropical regions of India. Numerous active substances, including flavonoids, saponins, flavonoids, quinones, phenols, sterols, triterpenoids, glycosides, tannins, and trace amounts of sugars, are found in the leaves of this plant. Pinitol, a hypoglycemic substance with insulin-like effects, is present in the leaf extract of this plant. One of the key underlying mechanisms of *Bougainvillea spectabilis* antidiabetogenic effect is thought to be its glucosidase inhibitory activity against mouse pancreatic and intestine glucosidase. Although antioxidant activity has been demonstrated in extracts from leaves of bougainvillea, only a limited number of tests have been performed for its determination. (14-16).

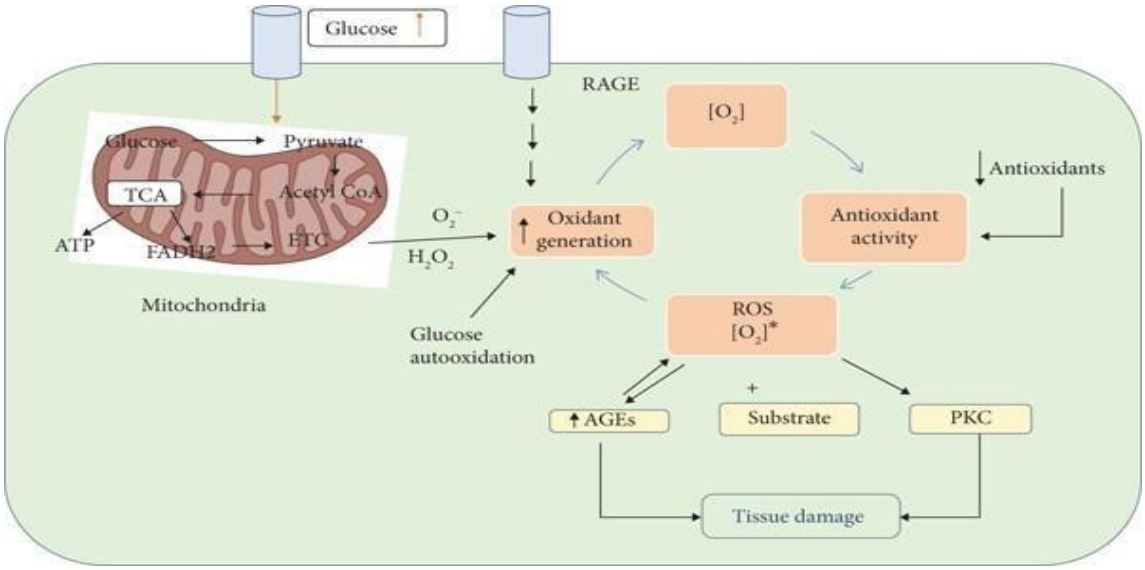

Figure 3: Mechanism of free radical generation

II. MATERIALS AND METHODS

To achieve the desired outcome while following established rules and standards, it's necessary to carry out a series of systematic actions when establishing a methodology. It involves the selection and gathering of medicinal plants, the choice of extraction solvents, the choice of dose, the standardization of the procedure, the use of instruments, the fabrication of reagents, the formulation of the protocol, and its ultimate implementation.

A. Plant collection and drying

The leaves and flowers of *Bougainvillea spectabilis* wild was collected from Hyderabad district, Telangana. After being dried in the shade for approximately six days, the leaves are ground into a rough powder using a mixer grinder. The powdered material was either stored or extracted using a specific procedure.

B. Preparation of plant extract

Principle

Utilizing siphon and reflux, soxhlet extraction is a practical and affordable technique. The continual extraction of solid material using a pure solvent is a key component of the concepts. On thimble-shaped filter paper, a solid sample is placed in the Soxhlet device before being processed. The soluble portion of the sample mixes with the solvent during heating of the reservoir flask, which is then used to extract the sample from it. Until the extracted substance is concentrated, the extract-containing solvent is siphoned back and the procedure is repeated many times.

Procedure

A glass cylinder was used to store 500g of unprocessed plant powder, which was first deposited on filter paper before being put inside. Both an intake tube and a siphon tube are present in the cylinder. The top of the cylinder is also attached to a water condenser. This arrangement is then put into the neck of a flask with a circular bottom and methanol as the solvent. To stop solvent from bumping, porcelain bits are put into the flask. Heat the flask in a water bath while allowing solvent vapors to enter through the intake tube to dissolve an organic material. The organic material is dissolved by the resultant liquid once these vapors condense in the condenser. A constant supply is maintained as additional solvent vapors enter the cylinder as the solution rises to the top of the siphon tube. Back into the flask flows the dissolved organic component. Once complete, turn off the heat and distill the mixture to remove the solvent, leaving the organic component behind.

C. *In vitro* anti-diabetic activity

1. α-amylase inhibition assay

20 mM phosphate buffer (pH 6.9) included 0.5 mg/ml of alpha amylase that was dissolved to create the enzyme solution. One milliliter of an enzyme solution was combined with various doses of leaf extract and standard (acarbose) (250, 500, 750, and 1000 g/ml) The solution mixture was heated to 25 ℃ and incubated there for 10 minutes. After the solution mixture had been incubated, 1 ml of starch solution (0.5%) was added, which underwent a further 10 minutes of incubation at 25 °C. To stop the process, 2,5-dinitro salicylic acid was added to 2 ml of coloring solution. The reaction mixture was warmed for five minutes in a bain-marie of boiling water. After cooling, the absorbance at 565 nm was determined colorimetrically (19, 20).

2. α-glucosidase inhibition assay

For 5 min at 37°C, different dosages of plant extract were added to a solution of starch substrate (2% w/v maltose or sucrose) in 1 ml of 0.2 M Tris buffer pH 8.0 to determine the inhibitory activity. The reaction was initiated by the addition of alpha-glucosidase enzyme (1 U/ml) and incubated at 37 °C for 10 minutes. Then, for two minutes, the reaction mixture was heated in a bath of boiling water to stop the process. Using the glucose oxidase peroxidase technique, released glucose is quantified (21-23).

Calculation of 50% Inhibitory Concentration (IC50):

The concentration of plant extracts required to scavenge 50% of the radicals (IC50) was calculated using the percentage scavenging activities at five different extract concentrations. I% = (Ac-As)/Ac X 100 (24). This formula was used to calculate the percentage of inhibition (I%).

Where As is the sample's absorbance and Ac is the absorbance of the control.

D. *In vitro* antioxidant activity

1. Hydroxyl radical scavenging activity

The ability of the extractives to scavenge hydroxyl radicals was evaluated by applying the method outlined by Halliwell et al. (25). The hydroxyl radical was created using the Fe3+-ascorbate-EDTA-H2O2 reaction system. The test relies on the determination of the byproduct of 2-deoxy-D-ribose breakdown, which becomes pink when heated with TBA at a low pH. The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L1, pH 7.4), 0.2 mL of extractives or standards at varied concentrations (12.5-150 g/mL), 0.2 mL of EDTA (1.04 mmol L1), 0.2 mL of FeCl3, and 0.2 mL of 2-deoxy-D-ribose (28 mmol L1). The combinations were remained immersed in a water bath at 37 °C and the reaction was initiated by adding 0.2 mL of ascorbic acid (AA). After being incubated at 37 °C for 1 hour, the reaction mixture was treated with 1.5 mL of cold thiobarbituric acid (10 g L1) and 1.5 mL of HCl (25%), respectively. Prior to cooling with water, the mixture was heated at 100 °C for 15 minutes. The solution's absorbance was measured using a spectrophotometer at 532 nm. To evaluate the capacity to scavenge hydroxyl radicals, it was necessary to reduce the proportion of 2-deoxy-D-ribose oxidation on the radicals.

The formula used to determine the percentage of hydroxyl radical scavenging activity is as follows:

% hydroxyl radical scavenging activity = [A0(A1A2]100/A0

where A0 represents the control's absorbance in the absence of a sample. The absorbance at the sample and 2-deoxy-D-ribose addition is A1. The sample's absorbance without 2-deoxy-D-ribose is A2. After that, the concentration was plotted against the percentage of inhibition, and the IC50 was determined from the graph. Three times at each concentration, the experiment was repeated.

2. Ferrous reducing antioxidant capacity assay

The samples' ferrous-reducing antioxidant capacity (FRAC) was determined using Oyaizu's method (26). The Fe2+ may be seen by keeping an eye on the Perl's Prussian Blue output at 700 nm. The test tubes contained 0.25 mL of samples or standard (aminoguanidine) solution at different concentrations (12.5-150 g/mL), 0.625 mL of potassium buffer (0.2 M), 0.625 mL of 1% potassium ferricyanide [K3Fe (CN)6] solution, and 0.625 mL of each solution. The reaction mixtures were incubated for 20 minutes at 50 °C to finish the reaction. After that, 0.625 mL of a 10% solution of trichloroacetic acid (TCA) was added to the test tubes. The centrifuged mixture's supernatant, which weighed 1.8 mL, was blended with 0.36 mL of a 0.1% ferric chloride (FeCl3) solution, 1.8 mL of distilled water, and 1.8 mL of the mixture. At 3000 rpm for 10 minutes, the entire mixture was centrifuged. The solution's absorbance at 700 nm in contrast to a control was measured using a spectrophotometer. The standard blank solution, which was incubated under the same conditions but did not include any standards or plant extracts, was prepared from the same solution combination. The absorbance was measured at 700 nm for the blank solution. The improved reducing power of the reaction mixture is shown by the reaction mixture's increased absorbance. The experiment was performed three times at every concentration.

III. RESULTS

Preliminary phytochemical screening: An first phytochemical analysis of MEBS found that it included a variety of phytoconstituents, including phenolic chemicals, alkaloids, flavonoids, saponins, terpenoids, tannins, carbohydrates, and others.

Table 1: Alpha amylase inhibtory activity of *Bougainvillea spectabilis*

S. No	Compound	Concentration (µg/mL)	% inhibition	IC_{50} value (µg/mL)
1	MEBS	20	32.5	70.58
2		40	40.2	
3		60	48.3	
4		80	52.0	
5		100	60.5	
6	Acarbose	20	42.0	36.3
7		40	56.5	
8		60	63.2	
9		80	72.3	
10		100	85.6	

According to Table 1, the % inhibition against alpha amylase activity increased with concentration in the crude extract of B. *spectabilis* leaves. At doses of 20, 40, 60, 80, and 100 g/ml, respectively, the crude extract showed a percentage inhibition of 32.5%, 40.2%, 48.3%, 52.0%, and 60.5%, reaching an IC50 of 70.58 g/ml.

Table 2: Alpha glucosidase inhibtory activity of *Bougainvillea spectabilis*

S. No	Compound	Concentration (µg/mL)	% inhibition	IC_{50} value (µg/mL)
1	MEBS	20	30.5	76.90
2		40	38.5	
3		60	45.4	
4		80	49.7	
5		100	58	
6	Acarbose	20	36.2	58.60
7		40	45.3	
8		60	52.4	
9		80	64.3	
10		100	76.4	

According to table 2, the % inhibition against alpha glucosidase activity increased with concentration in the crude extract of B. *spectabilis* leaves. At doses of 20, 40, 60, 80, and 100 g/ml, respectively, the crude extract showed a percentage inhibition of 30.5%, 38.5%, 45.4%, 49.7%, and 58%, reaching an IC50 of 76.90 g/ml.

Table 3: Hydroxyl radical scavenging assay of the extract and standard

S. No	Compound	Concentration (µg/mL)	% inhibition	IC_{50} value (µg/mL)
1	MEBS	10	26.5	24.0
2		20	47.2	
3		30	62.3	
4		40	70.2	
5		50	74	
6	Ascorbic acid	10	28.7	19.76
7		20	50.6	
8		30	64.4	
9		40	72.6	
10		50	74.8	

According to table 3, the percentage inhibition against the hydroxyl radical scavenging assay increased with concentration in the crude extract of B. *spectabilis* leaves. At doses of 10, 20, 30, 40, and 50 g/ml, respectively, the crude extract showed a percentage inhibition of 26.5%, 47.2%, 62.3%, 70.2%, and 74%, reaching an IC50 of 24.0 g/ml.

Table 4: FRAP assay of the extract and standard

S. No	Compound	Concentration (µg/mL)	% inhibition	IC_{50} value (µg/mL)
1	MEBS	10	26.3	24.5
2		20	46.2	
3		30	61.0	
4		40	69.2	
5		50	75.6	
6	Ascorbic acid	10	27.5	20.49
7		20	48.8	
8		30	62.9	
9		40	71.6	
10		50	78.3	

According to table 4, the percentage inhibition against the FRAP test increased when the concentration of the crude extract of B. *spectabilis* leaves was increased. At doses of 10, 20, 30, 40, and 50 g/ml, respectively, the crude extract showed a percentage inhibition of 26.3%, 46.2%, 61.0%, 69.2%, and 75.6%, reaching an IC50 of 24.5 g/ml.

IV. DISCUSSION

Inhibiting the activities of the intestinal pancreatic enzymes α-glucosidase and α-amylase lowers postprandial hyperglycemia (27). The reason for this is because absorbable monosaccharides have delayed carbohydrate digestion. In the current study, the crude B. spectabilis extract demonstrated -amylase inhibitory activity. Finding new -amylase inhibitors from medicinal plants is a noteworthy method for treating postprandial hyperglycemia.

Secondary metabolites including tannins, phenolic acids, and flavonoids have an inhibitory effect on -amylase that makes them the main phytoconstituents (28). A substantial quantity of polyphenolic compounds with -amylase inhibitory effect is present in crude extract, according to a previous finding (29).

Additionally, it is possible to hypothesize that the crude extract included larger levels of phytoconstituents for α-amylase inhibition. Flavonoids, tannins, lignans, phenolic acids, coumarins, lignins, and stilbenes are only a few examples of the many phenolic chemicals that are often abundant in medicinal plants (30). One of the treatment methods for treating DM to lower postprandial hyperglycemia (delaying the absorption of glucose) is inhibition of α-glucosidase. The treatment of DM has included the use of alpha-glucosidase inhibitors, which account for a sizeable share of the anti-diabetic drug market (31).

Oxidative stress is brought on by the body's increased generation of reactive oxygen/nitrogen species and diminished ability to fend off antioxidant attacks (32, 33). For aerobic organisms and healthy cells, the production of reactive oxygen/nitrogen species (ROS/RNS) is unavoidable and happens at a regulated pace (34). As a result of the substantial rise in ROS/RNS production under oxidative stress, membrane lipids, proteins, and nucleic acids are changed. Ageing and a number of pathogenic processes, such as atherosclerosis, cancer, ischemia reperfusion injury, and neurological illnesses, are linked to the oxidative degradation of these biomolecules (35).

This study also investigated the hydroxyl radical scavenging activity of plant extracts. Research has shown that the hydroxyl radicals directly denature body enzymes by oxidizing thiol (-SH) groups [42]. The hydroxyl radicals are created by the Fenton reaction as follows: $Fe^{2+} + H_2O_2 \; Fe^{3+} + OH + OH^{\bullet}$ (43). If a compound can scavenge for hydroxyl radicals in vitro, it is thought to be a strong antioxidant with potential advantages in vivo. In the current experiment, the FRAP test—ferric reducing antioxidant power—was employed. This approach relies on the crude extract's capacity to convert ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) (36, 37). As a result, the absorbance capacity at 700 nm may be used to analyze the Fe^{2+} formation (38). An increase in reducing power is shown by increases in absorbance at this wavelength (39).

The results of this investigation showed that the methanolic extracts of *Bougainvillea spectabilis* increased in absorbance values in a concentration-dependent manner, indicating significant ferric reducing antioxidant potential.

V. CONCLUSION

Modern medications often have plant-based roots. In light of the rising popularity of herbal medicines, it's critical to search for fresh anti-diabetic components in plant extracts. Based on the results, it is clear that the plant is a good candidate for the creation of novel anti-diabetic medications for the treatment of diabetes. The methanolic extract of *Bougainvillea spectabilis* has exhibited substantial anti-diabetic and antioxidant activity.

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