Inhibitory Effects of Methanol Stem Bark Extracts of Sterculia Setigera and Ficus Platyphylla on Hemoglobin Glycosylation and α-amylase

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Abstract  Diabetes Mellitus is a chronic metabolic disorder characterized by hyperglycemia due to abnormal insulin secretion, action or both. The synthetic available anti-diabetic drugs exhibited various adverse effects such as diarrhea, hypoglycemia. In recent years, scientists have turned their attention towards the medicinal plants which bear the rich source of metabolites which offer specific therapeutic function in the human body without any adverse effect. Sterculia setigera and Ficus platyphylla are medicinal plants that are used to treat various diseases and including diabetes traditionally. The present study is aimed at investigating the antidiabetic activity of the S. setigera and F. platyphylla methanol stem bark extracts. Phytochemical screening was determined using the standard method. In vitro studies were carried out using α-amylase and glycosylated hemoglobin inhibitory assay. The results of phytochemical constituents detected were flavonoids, Tannins, Steroids, Saponins, cardiac glycosides, Terpenoids, and Phenols. Inhibitory effects of both plant extracts were dose dependent against haemoglobin glycosylation and α-amylase. At concentration of (1.0mg/ml) S. setigera (72.21%) and F. platyphylla the (70.41%) showed the highest inhibitory effect which was not significantly different (p<0.05) compared to Voglibose (83.47%). In the present study, the IC₅₀ of both extracts were higher (0.64 and 0.69mg/ml) and not significantly comparable to the Voglibose (0.26mg/ml). In conclusion, this study suggests that Sterculia setigera and Ficus platyphylla methanol stem bark extract possess hypoglycaemic potentials. This justifies their ethnomedicinal use for the treatment of diabetes.

Keywords  Ficus Platyphylla, Sterculia Setigera, Antidiabetic, α-amylase, Haemoglobin

1. Introduction  Diabetes mellitus could also be a set of prolonged metabolic disorders characterized by hyperglycemia as a result of defects in insulin secretion, action, or both [1]. The increasing incidence of diabetes mellitus worldwide among all age groups irrespective of sex, race, socioeconomic status, or ethnicity, constitutes a global public health burden [1-3]. Clinically, we have two major types of diabetes mellitus (DM), type1 DM represents
insulin dependent diabetes mellitus as a result of acute or chronic insulin deficiency in plasma and accounts for 5–10% of all cases of diabetes mellitus and is caused by the autoimmune destruction of pancreatic β-cells which results in insulin deficiency [6]. On the opposite hand, Type 2 DM (T2DM) is one among the foremost common metabolic disorders worldwide and its development is primarily caused by a mixture of two main factors: defective insulin secretion by pancreatic β-cells and therefore the inability of insulin sensitive tissues to reply to insulin [24].

Globally, about 415 million individuals were affected with diabetes in 2015 and this has been projected to extend to 629 million by 2045 [5]. Previous report revealed that prevalence of DM in Nigeria has increased from 2.2% in 1997 to 5.0% by 2013 [4]. The high costs and adverse effects of insulin and therefore the available oral hypoglycaemic agents have necessitated increased investigations on medicinal plants used ethnomedically for the management of diabetes [8-9]. According to the WHO, over 80% of the world’s population depend upon traditional sorts of medicine, largely plant based to satisfy primary health care needs [18]. Medicinal plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world [19]. The present study was designed to investigate the antidiabetic activities of *Sterculia setigera* and *Ficus platyphylla* methanol stem bark extract with a view to justifying its antidiabetic folkloric claims.

### 2. Materials and Methods

#### 2.1. Materials

**2.1.1. Chemical and Reagents**

All chemicals used were of analytical grade.

**2.1.2. Equipments/ Instrumentation**

- Spectrophotometer, rotary evaporator, incubator, test tube, beaker, cuvette, test tube rack, syringe

**2.1.3. Plants Collection and Identification**

Fresh stem bark of *Sterculia Setigera* and *Ficus Platypylla* were collected in November, 2020 at local farmlands Zuru Local Government Area, Kebbi State. It was identified and authenticated by a Taxonomist at the Botany Unit department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. Voucher specimens (KSUSTA/PSB/H/VOUCHER NO: 29A and KSUSTA/PSB/H/VOUCHER NO: 83B) have been deposited in the Herbarium of the same institution for further reference.

**2.1.4. Anti-diabetic Conventional Drug**

Voglibose (Macrolabs, INDIA) and Metformin (Sanofi Aventis, INDIA) were purchased in December, 2020 from Hamdala Pharmacy Birnin Kebbi, Kebbi State.

#### 2.2. Methods

**2.2.1. Preparation of Plant Material**

The stem bark of both plants was shade dried at room temperature for seven days to liberate moisture content and reduce to a constant weight. Dried stem bark of both plants were powdered individually in a clean mortar and pounded with a pestle. The powdered material was sieved and stored in a sterile, tight container until needed. Two hundred fifty grams (250g) of each powdered plant material was weighed and subjected to cold maceration using 1250L of methanol and was allowed to stand for 75 hours with occasional turning and shaking. The mixture was filtered afterward with a clean white Muslim cloth. The filtrate obtained was evaporated to dryness using a rotary evaporator at 45°C [10].

**2.3. Qualitative Phytochemical Screening**

**2.3.1. Test for Alkaloids (Dragendroff’s Test)**

About 0.5g of each plant extracts was stirred with 5ml of 1% dilute hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of reagent, and a second 1ml of extract was treated with Dragendroff’s reagent. Presence of turbidity or precipitation is evidence for the presence of alkaloids [31].

**2.3.2. Test for Flavonoid (Alkaline Reagent Test)**

Few drops of Sodium hydroxide were added to 5ml of each extracts, a yellow coloration.

**2.3.3. Test for Tannins (Ferric Chloride Test)**

About 0.5ml of each plant extract was stirred with 5ml of distilled water and then filtered and 23 drops of ferric chloride reagent was added to the filtrate. Black or blue-green precipitate was taken as evidence for the presence of tannins [31].

**2.3.4. Test for Saponins (Frothing Test)**

Exactly 5ml of each plant extracts in a test tube, 5ml of distilled water was added to each test tube and shaken strongly. The formation of froth that lasted for several minutes is an indication of the presence of saponins (Harbone, 1973).

**2.3.5. Test for Cardiac Glycosides (Keller- Mililani’s Test)**

2ml of each plant extract was added to 2ml of 3.5% Ferric chloride solution in two different test tubes and was allowed to stand for a minute. About 1ml of concentrated H2SO4 was carefully poured right down to the wall of the tube to make a lower layer. A reddish-brown ring at the interface indicated the presence of cardiac glycosides [28].
2.3.6. Test for Terpenoids (Salkowski Test)

2.5ml of both plant extract was mixed with 1ml of chloroform and 1.5ml concentrated H2SO4 was carefully added to make a layer during a different tube. A reddish-brown coloration at the interface indicates a positive result for the presence of terpenoids [31].

2.3.7. Test for Phenolic Compound

2ml of methanol stem bark extract of Sterculia setigera and Ficus platyphylla was mixed with 2ml of ferric chloride solution and the mixture was shaken individually. Dark green colour indicates the presence of the phenolic compound of phenols [12].

2.3.8. Test for Carbohydrates (Molisch’s Test)

20mg of both plant extracts was dissolved in 5ml distilled water differently and filtered. The filtrate was treated with 2 drops of alcoholic α-naphthol solution during a tube. The formation of the violet ring at the junction indicates the presence of Carbohydrates [31].

2.4. In vitro Antidiabetic Assay

2.4.1. Non-enzymatic Glycosylation of Haemoglobin Inhibitory Assay

1ml of Glucose (2%), haemoglobin (0.06%) and Gentamicin (0.02%) was prepared in a phosphate buffer 0.01M at pH 7.4 each and mixed together. Then methanol extracts of both plants were weighed and dissolved in distilled water to obtain various concentrations of 525mg/ml of (SSME and FPME). Then 1ml of each concentration of plant extracts was added to the above mixture. The mixture was incubated in darkness at room temperature for 72 hrs. The inhibitory effect of plant extracts on glycosylation of haemoglobin was measured using spectrophotometric at 443nm [13]. Metformin was used as a standard drug for assay and % inhibition was calculated using the formula:

\[
\text{Percentage Inhibition} = \left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}}\right) \times 100
\]

\{Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample and The IC50 values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined using (ic50.tk/index.html)\}.

2.4.2. α-Amylase Inhibitory Assay

In the α-amylase inhibitory method, the enzyme solution was prepared by dissolving 50mg of α-amylase in 100ml of 20mM phosphate buffer (6.9) and obtaining concentration of 0.5mg/ml. 1ml of various concentrations (0.2, 0.4, 0.6, 0.8 and 1mg/ml) of both plant extracts was mixed with 1ml of enzyme solutions and incubated at 25°C for 10min. After incubation, 1ml of starch (1%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was stopped by adding 2ml of dinitrosalicylic acid (DNS, color reagent), heating the reaction mixture in a boiling water bath for five (5min). After cooling, the absorbance of the mixture was measured spectrometrically at 540 nm [23].

The inhibition percentage was calculated using the given formula,

\[
\text{Percentage Inhibition} = \left(\frac{\text{Abs Sample} - \text{Abs Control}}{\text{Abs Sample}}\right) \times 100
\]

\{Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample and The IC50 values (inhibitory concentration which will produce 50% inhibition of the enzyme activity) of the plant extracts were determined using (ic50.tk/index.html)\}.

2.5. Statistical Analysis

The data were expressed as mean ± S.E.M. The significance of the difference of the mean value for both standard drug and that of plant extract was analyzed using one-way ANOVA followed by Duncan multiple comparison test. p<0.05 was as considered to be significantly different.

3. Results

3.1. Results of Phytochemical Screening

The qualitative phytochemical screening of Sterculia setigera and Ficus platyphylla methanol stem bark extract is presented in (Table 1). The results for the phytochemical screening of Sterculia setigera revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Carbohydrates, Cardiac glycoside, Phenols and Terpenoids.

Table 1. Qualitative Phytochemical Constituents of Sterculia setigera and Ficus platyphylla Methanol Stem Bark Extract.

| PHYTOCHEMICALS      | S. SETIGERA | F. PLATYPHYLLA |
|---------------------|-------------|----------------|
| ALKALOIDS           | -           | -              |
| FLAVONOIDS          | +           | +              |
| TANNINS             | +           | -              |
| SAPONIN             | +           | +              |
| CARBOHYDRATES       | +           | +              |
| CARDIAC GLYCOSIDE   | +           | +              |
| PHENOLS             | +           | +              |
| TERPENOIDS          | +           | +              |

KEYS: + = Present, - = Not Detected
3.2. Effect of *F. platyphylla* and *S. setigera* on Haemoglobin Glycosylation

There was a dose-dependent increase in percentage inhibitory effect against hemoglobin glycosylation over the period of 72 hrs, as the concentration of plant extracts increases (Table 2). This suggests that the plant extracts decrease the formation of the glucose-haemoglobin complex and thus the amount of free haemoglobin increases. At highest concentration (25mg/ml) highest inhibitions were recorded in *S. setigera* (70.30%) and *F. platyphylla* (70.00%) which was comparable to Metformin (57.2%). IC$_{50}$ of *Sterculia setigera* (3.18mg/ml) and *Ficus platyphylla* (5.97mg/ml) were lower than metformin (8.84 mg/ml) indicating that the plant extracts is more potent than standard drug against hemoglobin glycosylation.

### Table 2. Haemoglobin Glycosylation Inhibitory Effect of *F. platyphylla* and *S. setigera* Methanol Stem Bark Extracts

| Period | Conc. mg/ml | Metformin | *S. setigera* | *F. platyphylla* |
|--------|------------|-----------|---------------|------------------|
| 24 hrs |            |           |               |                  |
| 5      | 2.37±0.01  | 6.00±0.02 | 20.00±0.02    |                  |
| 10     | 8.13±0.01  | 38.67±0.01| 27.70±0.03    |                  |
| 15     | 15.15±0.01 | 46.25±0.15| 33.30±0.05    |                  |
| 20     | 25.50±0.01 | 48.20±0.02| 34.90±0.05    |                  |
| 25     | 33.60±0.02 | 48.20±0.02| 38.80±0.06    |                  |
| 48 hrs |            |           |               |                  |
| 5      | 13.60±0.01 | 48.90±0.01| 43.70±0.05    |                  |
| 10     | 31.30±0.01 | 61.10±0.09| 50.10±0.02    |                  |
| 15     | 34.60±0.01 | 62.70±0.05| 60.30±0.03    |                  |
| 20     | 34.60±0.04 | 63.50±0.03| 62.50±0.02    |                  |
| 25     | 34.80±0.01 | 65.00±0.17| 65.40±0.01    |                  |
| 72 hrs |            |           |               |                  |
| 5      | 17.71±0.01 | 55.49±0.05| 56.51±0.03    |                  |
| 10     | 36.33±0.01 | 68.10±0.02| 63.03±0.01    |                  |
| 15     | 44.53±0.02 | 68.5±0.03  | 66.27±0.03    |                  |
| 20     | 55.03±0.20 | 69.40±0.03 | 68.76±0.01    |                  |
| 25     | 57.52±0.04 | 70.30±0.04| 70.00±0.03    |                  |
| IC$_{50}$ | 8.84 | 3.18 | 5.97 |

Values are expressed as mean ± SEM of n=3

3.3. α-Amylase Inhibitory Effects of *F. platyphylla* and *S. setigera* Methanol Stem Bark Extracts

A dose-dependent increase in α-Amylase inhibition was exhibited by *Sterculia setigera* and *Ficus platyphylla* as their concentration increases (Table 3). At concentration of (1.0mg/ml) *S. setigera* (72.21%) and *F. platyphylla* (70.41%) showed the highest inhibitory effect which was not significantly different (p<0.05) compared to Voglibose (83.47%). In the present study, the IC$_{50}$ of both extracts were higher (0.64 and 0.69mg/ml) and comparable to the Voglibose (0.26mg/ml) suggesting that the voglibose is more potent than both extracts.

### Table 3. α-amylase Inhibitory Effect of *F. platyphylla* and *S. setigera* Methanol Stem Bark Extract

| Conc. mg/ml | % inhibition (mean ± SEM) | *S. setigera* | *F. platyphylla* | Voglibose |
|------------|---------------------------|---------------|------------------|-----------|
| 0.2        | 14.07±0.02$^d$           | 16.52±0.01$^d$| 17.48±0.00$^d$   |
| 0.4        | 19.03±0.03$^c$           | 17.96±0.01$^d$| 48.38±0.01$^d$   |
| 0.6        | 44.01±0.02$^b$           | 33.00±0.02$^c$| 60.59±0.05$^c$   |
| 0.8        | 58.20±0.03$^b$           | 58.20±0.04$^a$| 70.65±0.04$^b$   |
| 1.0        | 72.21±0.02$^a$           | 70.41±0.03$^c$| 83.47±0.00$^c$   |

Values are mean ± SEM of triplicate determinations. Values having different superscripts are significantly different (p <0.05)

3.4. Discussion

Phytochemicals obtained from medicinal plants offer a promising alternative for the development of new therapeutic agents against diabetes mellitus [14]. The use of medicinal plants has a long folkloric history for the treatment of diabetes mellitus as well as oxidative stress related conditions [33-34]. Secondary metabolites such as Flavonoids, Steroids, Triterpenoids, Saponins, Alkaloids, and Phenolic have been reported to possess anti diabetic activities [27, 30]. This activity might be achieved by stimulating insulin release from pancreatic β-cells, inhibiting glucose absorption in the gut, inhibiting carbohydrates digesting enzymes, stimulating glycogenesis in the liver and/or increasing glucose utilization by the body [16]. The hypoglycemic effects observed in the present study, of both plant extracts may be due to the presence of such phyto-constituents in the plant extracts.

In *vitro* non-enzymatic glycosylation of hemoglobin method is one of the important assays to evaluate the control of diabetes [22]. The hemoglobin attached to RBCs has an affinity to bind to glucose. The greater the glucose level in the blood the higher the amount of glucose hemoglobin complex will be formed [7, 22]. Consequently, the presence of a higher concentration of glycosylated hemoglobin is a sure guide to the higher concentration of glucose in the blood. Normal percentage of Glycated hemoglobin should not exceed 12% in the human blood [32]. The current study revealed that both plant extracts exhibited good inhibitory activities on glucose-hemoglobin complex formation by preventing such complexes (that is glucose binds to the surface of proteins). This is in corroboration with the previously reported research on different plant extracts [11, 15, 17, 21, 26, 32].
α-amylase enzyme is one of the enzymes responsible for the hydrolysis of α-oriented bond polysaccharides and oligosaccharides such as starch, glycogen, and other macromolecules of α bond linked monosaccharides to disaccharides and finally, to glucose [29]. It is well known that the reduction of postprandial hyperglycemia can be achieved by inhibiting intestinal α-glucosidase and pancreatic α amylase activity via delayed carbohydrate digestion [20]. It is reported that when α amylase, glucose, plant extract are taken together as a solution, the plant extract causes the inhibition of the enzyme activity [35]. In the present study, the potent inhibitory activities of Sterculia setigera and Ficus platyphylla methanol stem bark extracts observed suggest that both plants possess the antidiabetic effects. These inhibitory potentials may be ascribed to the presence of phytochemical constituents such as flavonoids, tannins, and Saponins which have also been reported previously to inhibit α-amylase activity [25].

4. Conclusion

In conclusion, S. setigera and F. platyphylla stem bark extract produced inhibitory effects against haemoglobin glycosylation and α-amylase validating hypoglycemic potentials of both extracts. This supports ethnopharmacological use of these plants in the management of diabetes.

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