

**Evaluation of the Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of *Stachytarpheta jamaicensis* (Verbenaceae)**Ewaen Egharevba¹, Patience Chukwuemeke-Nwani¹, Uche Eboh¹, Esther Okoye¹, Israel Olapeju Bolanle², Irene O. Oseghale³, Vincent O. Imieje³, Osayemwenre Erharuyi^{3*}, Abiodun Falodun³¹Department of Biological Sciences (Biochemistry Unit), Faculty of Science, Benson Idahosa University, Benin City, Edo State, Nigeria.²Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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ABSTRACT

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Stachytarpheta jamaicensis (family Verbenaceae) commonly known as blue porterweed is widely used in folk medicine for the treatment of various diseases including diabetes mellitus. The present study investigated the blood glucose lowering effect and the antioxidant activity of the leaf extracts of *Stachytarpheta jamaicensis*. The phytochemical screening of the powdered leaf sample was done according to standard procedures. The antioxidant activity of the methanol and ethyl acetate extracts of the leaves were investigated using the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay, the total phenolic and flavonoid contents were also evaluated following standard procedures. The hypoglycaemic effect of the extracts was evaluated using streptozotocin-induced diabetes in rats.

Phytochemical screening shows the presence of carbohydrates, alkaloids, saponins, tannins, phenolic compounds, and flavonoids. The extracts demonstrated appreciable and concentration-dependent radical scavenging effect with IC₅₀ values of 16.95 µg/mL and 33.12 µg/mL, for the methanol extract and ethyl acetate extract, respectively. Oral administration of the extracts at 200 mg/kg and 400 mg/kg daily dose significant lowers blood glucose levels in streptozotocin-induced diabetes in experimental rats compared to the control group (untreated diabetic animals). The present findings have therefore shown that *S. jamaicensis* leaves has hypoglycaemic and antioxidant effects and may therefore serve as a potential source of hypoglycaemic agent as well as antioxidant agents for the prevention and management of free radical induced metabolic diseases.

Keywords: *Stachytarpheta jamaicensis*, Diabetes, Herbal medicines, Free radicals, Antioxidants.

Introduction

Diabetes mellitus (DM) is a syndrome with abnormalities in carbohydrate, lipid and protein metabolism. The central disturbance in DM is a derangement in insulin production or action or both, although other factors can be involved. Hyperglycaemia is commonly the end point for all types of diabetes mellitus and is the factor that is measured to evaluate and manage the efficacy of diabetes therapy.¹ DM is a disease of gradual onset and the symptoms, when they eventually appear, do not provoke immediate attention and thus can remain undiagnosed at onset and even when diagnosed is usually not given attention by persons afflicted by the disease.² DM is typically not reversible and, although patients can have a reasonably normal lifestyle, its late complications can cause a reduced life expectancy and major health cost. These include microvascular assault causing diabetic nephropathy, neuropathy and

*Corresponding author. E-mail: osayemwenre.erharuyi@uniben.edu
Tel: +2348062355972

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retinopathy, and macrovascular complications, leading to an increased prevalence of coronary artery disease (CAD), peripheral vascular disease and cerebrovascular accident.³

The etiological classification of diabetes has now been widely accepted; type 1 and type 2 are the two main types. Although the prevalence of both type 1 and type 2 DM is on the increase worldwide, the prevalence of type 2 diabetes is rising much more rapidly. Reports have shown that type 2 diabetes constitutes 85 to 95% of all diabetes in high-income countries.⁴ The introduction of insulin therapy represented a major breakthrough in type 1 diabetes management and recent developments which include whole pancreas transplant or pancreatic islet transplant, stem cell, gene therapy and islets encapsulation have all formed a beacon of hope for a better management of this disease condition. The management of type 2 diabetes has been based on drugs that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reduce hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrates (alpha glucosidase inhibitors) or improve insulin action (thiazolidinediones).

The World Health Organization (WHO) estimates that around 80% of the population in Africa use traditional medicines, with about 85% of traditional medicine involve use of plant extracts.⁵ This estimate implies a large dependence on herbal medicine. To better appreciate the dependence, there is an estimate that in Sub-Saharan Africa there is a ratio of one traditional healer for every 500 people, compared to only one medical doctor for every 40,000 people. This shows that the importance of herbal medicines in the life of Africans cannot be

overemphasized. The insurgence of interest of herbal medicines in Africa is based on many factors, including the increasingly expensive and unavailability of orthodox drugs to average income earners.⁶ The very inconsequential or no side effects with use of herbal medicines is another factor.⁷

In the reality that DM is typically a chronic condition with a significant health cost, there is a need to look at viable alternatives to long-standing pharmacologic anti-diabetic drugs with notable side effects.

As a possible alternative, *Stachytarpheta jamaicensis* is widely known for its high medicinal value in traditional and folk medicine in Nigeria. This plant has been reported to have pharmacological activity due to the presence of various bioactive compounds. In herbal medicine, *S. jamaicensis* itself has been known to demonstrate analgesic,⁸ anti-inflammatory,⁹ antihelminthic,¹⁰ diuretic, laxative, lactagogue, purgative, sedative, spasmogenic, vasodilator, vulnerary, and vermifuge properties¹¹ and claims of blood sugar lowering effect by some traditional healers.¹²

This study, therefore, investigated the hypoglycaemic effect viz-aviz the antidiabetic property of *S. jamaicensis* leaves.

Materials and Methods

Plant material and extraction

Stachytarpheta jamaicensis leaves were collected from Iguikhimwin town, Ovia North East Local Government Area, Edo State, Nigeria in January 2018. The plant was identified and authenticated in the Department of Plant Biology and Plant Technology, University of Benin, Edo State, Nigeria. The fresh leaves were removed from the aerial part and air-dried for 7 days. The dried leaves were powdered using mechanical grinder. The powdered sample was weighed and stored in an air-tight glass jar until required for use. The crude powdered sample (450 g) was extracted successively with 1.5 L each of ethyl acetate and methanol by maceration at room temperature for 3 days with frequent shaking. The residue was removed by filtration using filter paper (Whatmann no. 1: 11 µm). The extracts were concentrated to dryness at a room temperature to yield a solid mass free from solvent. The dried extracts were kept in clean glass jars and stored in the refrigerator at 4°C.

Phytochemical screening

Simple chemical tests were carried out on the crude powdered sample according to standard procedures to identify the phytochemical constituent.^{13,14}

Determination of antioxidant activity

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the ethyl acetate and methanol extracts of *Stachytarpheta jamaicensis* was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, the method of Jain *et al.*¹⁵ was adopted. A solution of 0.2 mM DPPH in methanol was prepared. 1.0 mL of this solution was mixed with 3 mL of extract in methanol containing 0.001 - 0.2 mg/mL of the extract. The mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability to scavenge DPPH was calculated from the following equation.

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / (A_0)] \times 100$$

Where A_0 = absorbance of DPPH radical + methanol

A_1 = absorbance of DPPH radical + sample extract / standard

The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration that can scavenge 50% of the DPPH radical.

Total phenolic content

The total phenolic content was determined according to the method described by Kim *et al.*¹⁶ The extract solution (0.5 mL) with concentration 1 mg/mL was added to 4.5 mL of distilled water and 0.5 mL of folin ciocalteau reagent (previously mixed with distilled water 1.10 v/v). The tubes were allowed to stand at room temperature for 5

minutes after which 5 mL of 7% sodium carbonate was added. This was incubated for 90 minutes at room temperature and the absorbance was measured using a spectrophotometer at 750 nm. Gallic acid was used as the reference compound to prepare a standard plot from six concentrations [12.5, 25, 50, 75, 100, and 150 µg/mL]. The total phenolic content was expressed as milligrams Gallic acid equivalents per gram of extract (mg GAE/g extract).

Total flavonoids content

The test was determined according to the method previously described by Ebrahimzadeh *et al.*¹⁷ The extract solution (0.5 mL of 1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%) was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured with a spectrophotometer at 415 nm. Standardized curve was prepared with quercetin in six different concentrations (12.5, 25, 50, 75, 100, 150 µg/mL). The result was expressed as milligram quercetin equivalent per gram of extract (mg QE/g extract).

Antidiabetic Activity

Animals

Male Wistar rats weighing 150 – 250 g were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were kept in plastic cages and housed at room temperature, humidity and light/dark circle (12 h/12 h). They were fed with rodent pellets and allowed free access to water. The bedding materials (wood shavings) of the cages were changed daily.

Ethical consideration

Ethical approval (Reference No. EC/FP/019/08) was granted by the animal ethics committee of the Faculty of Pharmacy, University of Benin, Benin City. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) revised in 2002.¹⁸

Induction of Diabetes Mellitus

The animals were fasted overnight and diabetes mellitus was induced by a single dose (40 mg/kg body weight) intraperitoneal injection of streptozotocin dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5). After administration the animals were allowed free access to feed and water. After 48 hours the animals were tested for diabetes using the Accu-Chek® Active glucometer (Roche, USA) and any animal with a blood sugar ≥ 200 mg/dL was considered diabetic.

Experimental Design

The animals were grouped into six groups of five rats each and treated for one week as follows;

Group 1: Diabetic animals treated with 200 mg/kg/day of methanol extract of *Stachytarpheta jamaicensis* leaves.

Group 2: Diabetic animals treated with 400 mg/kg/day of methanol extract of *Stachytarpheta jamaicensis* leaves.

Group 3: Diabetic animals treated with 200 mg/kg/day body weight dose ethyl acetate extract of *Stachytarpheta jamaicensis* leaves.

Group 4: Diabetic animals treated with 400 mg/kg/day body weight dose ethyl acetate extract of *Stachytarpheta jamaicensis* leaves.

Group 5: Diabetic animals treated with 5 mg/kg/day body weight dose of glibenclamide (Daonil®).

Group 6: Untreated diabetic animals.

Determination of Blood Glucose

The lateral tail vein was pricked using a sterile lancet. One droplet of the blood was placed on the glucose test strip and read using the

Accu-chek® Active glucometer (Roche, USA). Blood glucose was determined at 0 hr, 1 hr, 2 hr, 4 hr, 8 hr and 7th day of treatment.

Statistical Analysis

Results were expressed as mean \pm standard error of the mean (SEM) of four replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range tests using the Statistical Analysis System (SPSS Statistics 17.0). P values < 0.05 were regarded as significant.

Results and Discussion

Phytochemical constituents of *Stachytarpheta jamaicensis* leaves

The results on phytochemical screening of powdered leaf extracts of *Stachytarpheta jamaicensis* are shown in Table 1 below. Carbohydrates, alkaloids, saponins, phenolic compounds, and flavonoids were present in the powdered sample. The presence of these phytochemicals agrees with the earlier report of Liew and Yong,¹⁹ who identified additional phytochemicals like terpenoids, steroids, proteins and glycosides in the leaves of *S. jamaicensis*.

DPPH free radical scavenging activity

Figure 1 represents the percentage free radical scavenging activity of the methanol and ethyl acetate extracts of *S. jamaicensis* leaves.

Stable free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH) have been used to evaluate the radical scavenging activity of antioxidants. The DPPH radical scavenging activity is measured on the basis of its strong absorption band at 517 nm. In this antioxidant test method, the antioxidant donates hydrogen to reduce the stable DPPH radical to a non-radical diphenyl picrylhydrazine (DPPH-H) leading to a decrease in its absorbance.²⁰ The extent of the decrease in absorbance of DPPH is a measure of the antioxidant ability of the test antioxidant.

The result of the DPPH radical scavenging assay showed that *S. Jamaicensis* extracts have good DPPH radical scavenging effect with the percentage scavenging activity of 59.53 and 60.20% for methanol extract and ethyl acetate extract, respectively at 100 $\mu\text{g/mL}$. The radical scavenging effects for both extracts (methanol and ethyl acetate) were significantly ($P < 0.05$) lower than that of the standard antioxidant (ascorbic acid) used which gave a percentage scavenging activity of 85.62% at 100 $\mu\text{g/mL}$. In the present study, the methanol and ethyl acetate leaf extracts of *S. jamaicensis* showed a concentration-dependent increase in radical scavenging activity with maximum effect at 100 $\mu\text{g/mL}$. Further increase in the concentration of the extracts beyond 100 $\mu\text{g/mL}$ resulted in no appreciable increase in the scavenging activity (Figure 1). As shown in Table 2, the 50% inhibitory concentration (IC_{50}) for the methanol extract (16.95 $\mu\text{g/mL}$) was lower than the value obtained for the ethyl acetate extract (33.12 $\mu\text{g/mL}$). This suggests that the methanol extract has a better scavenging effect viz-a-viz a better antioxidant activity than the ethyl acetate extract.

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of *S. jamaicensis* leaves extract were determined using spectrophotometric method and the results were obtained from the equation of the calibration curve of gallic acid ($Y = 0.004x + 0.0387$, $R^2 = 0.9994$) for total phenolic content and quercetin ($Y = 0.0067x + 0.3649$, $R^2 = 0.9949$) for total flavonoid content.

Phenolics are strong antioxidant due to their reducing properties, they inhibit the oxidation of organic matter by transferring hydrogen ion from their hydroxyl group to free radicals that cause oxidation. Therefore, the phenolic content of plants has been shown severally as a proportional measure of the antioxidant activity of plant extracts.²¹⁻²⁵

In this study, the methanol extract of *S. jamaicensis* leaves was found to have a higher phenolic content than the ethyl acetate extract with phenolic contents of 78.77 ± 0.26 mgGAE/g extract and 66.23 ± 2.45 mgGAE/g extract for the methanol and ethyl acetate extract, respectively (Table 3). The higher contents of phenol in the methanol extract may be due to its polar nature compared to ethyl acetate because phenolic

Table 1: Phytochemical constituents of the powdered leaf of *Stachytarpheta jamaicensis*.

Phytochemical	Inference
Alkaloids	+
Carbohydrates	+
Reducing sugars	+
Saponins	+
Phenolics	+
Flavonoids	+
Tannins	-

Where; + sign shows the presence of component and - sign shows the absence.

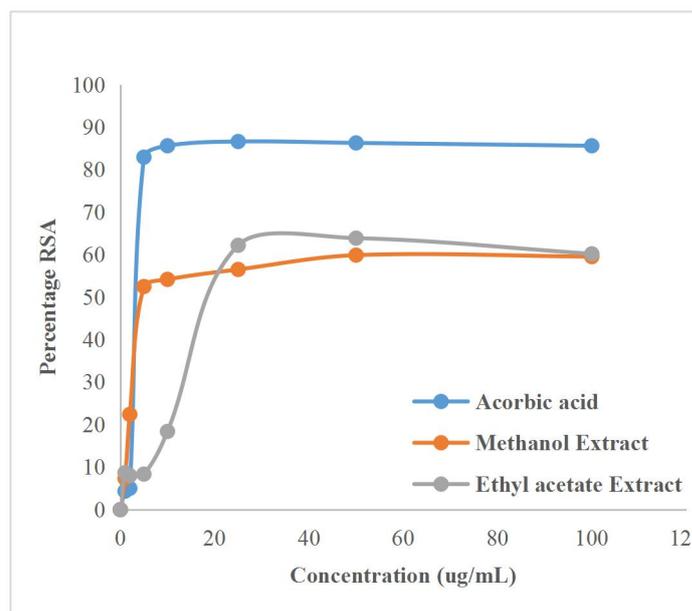


Figure 1: DPPH Radical Scavenging Activity of Extracts of *Stachytarpheta jamaicensis* leaves.

compounds being polar have been found to be present more in polar solvents compared to less polar solvents.

However, for the estimation of the total flavonoid content, the ethyl acetate extract of the plant had a higher flavonoid content (78.57 mgQE/g extract) compared to the methanol extract with a flavonoid content of 30.96 ± 2.64 mgQE/g extract.

Flavonoids are the most common polyphenolic compounds found mainly in fruits and vegetables. They have been reported to exert numerous biological activities including antioxidant activity.^{26,27} Bioflavonoids have been shown to have protective effect against hydroxyl radical-induced DNA damage,²⁸ and prevents brain damage due to diabetes mellitus.²⁹

Effect of extracts of *Stachytarpheta jamaicensis* on blood glucose level

The management of diabetes mellitus has remained a challenge globally.³⁰ With the increasing cost of orthodox medicine, there may be increased utilization of alternative medicines including herbs in the management of the disease. In the management of diabetes mellitus, a number of natural products including medicinal plants with antihyperglycaemic effect have been recognized and utilized.³¹ The phytochemical screening of *S. jamaicensis* leaf has reported the presence of carbohydrates, saponin, tannin, phenolic compounds, alkaloids,

Table 2: IC₅₀ value for DPPH Radical scavenging activity of *Stachytarpheta jamaicensis* leaf extract and ascorbic acid.

Sample	IC ₅₀ Value (µg/mL)
Ascorbic Acid	5.40
Methanol extract	16.95
Ethyl acetate extract	33.12

Table 3: Total phenolic content (TPC) and total flavonoid content (TFC) of extracts of *Stachytarpheta jamaicensis* leaves.

Sample	TPC	TFC
Methanol extract	78.77 ± 0.26	30.96 ± 2.64
Ethyl acetate extract	66.23 ± 2.45	78.57 ± 7.05

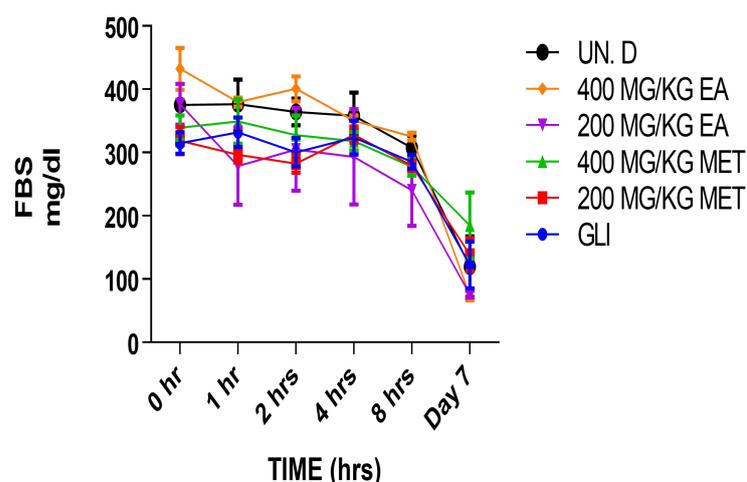
flavonoids, terpenoids, and steroids.¹⁸ These phytochemicals have been associated with a number of biological activities including; hypoglycaemic, anticancer, antimicrobial and antioxidants activities just to mention a few. In the present study, the mean value of the fasting blood glucose levels in streptozotocin-induced diabetic rats after daily administration of methanol and ethyl acetate extracts of *S. jamaicensis* leaf was shown to be significantly reduced compared to the control group. The results showed that the methanol leaf extracts at dose of 200 mg/kg showed a significant ($P < 0.05$) reduction in blood glucose level in streptozotocin-induced diabetic rat at the 1st hour following extract administration, with an abrupt increase at the 4th hour, after which there was a gradual decrease from the 4th hour up to the 7th day of administration. On the other hand, administration of 400 mg/kg of methanol leaf extract, resulted in a significant ($P < 0.05$) hypoglycaemic activity from the 1st hour up to the 7th day of the study when compared with that of the untreated diabetic control group (Figure 2).

Similarly, the ethyl acetate leaf extract of *S. jamaicensis* at dose of 200 mg/kg showed a significant reduction in blood glucose level in streptozotocin-induced diabetic rat at the 1st hour and an increase at the 4th hour followed by a decrease from the 4th hour up to the 7th day of administration, while the administration of 400 mg/kg of the ethyl acetate extract, also resulted in a significant hypoglycaemic activity from the 1st hour up to the 7th day of treatment. Glibenclamide the standard hypoglycaemic agent used in this study produced a significant hypoglycaemic activity at 0, 1st, 2nd, 7th and 8th day, while the untreated diabetic group showed no significant reduction in blood glucose level up until the 7th day of the treatment.

The use of experimental animal models of diabetes has helped in understanding its pathogenesis as well as testing the efficacy of novel drugs or herbal products. Some of the diabetogenic drugs in use include streptozotocin, alloxan monohydrate, ferric nitrilotriacetate, and antiinsulin serum. Studies have shown that streptozotocin induces diabetes by inducing DNA damage and destroying the beta cells of pancreas of the experimental animals, resulting in insufficient insulin secretion and consequently diabetes mellitus.³² In this study, significant hyperglycaemia of over 200 mg/dL (a threshold considered to be diabetic) was induced after streptozotocin injection.

Conclusion

This study has shown that *Stachytarpheta jamaicensis* possess hypoglycaemic effect and good antioxidant activity. The present observation demonstrated that *S. jamaicensis* may be a potential source of hypoglycaemic agent as well as antioxidant agent that can be used against free radical species, prevent diseases and may also act as a natural antioxidant in foods.

Data 2**Figure 2:** Effect of methanol and ethyl acetate extracts of the leaves of *Stachytarpheta jamaicensis* on fasting blood glucose levels in streptozotocin-induced diabetic rats.

MET = Methanol extract, EA = Ethyl acetate extract, GLI = Glibenclamide, UN.D = Diabetic untreated (Control).

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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