

Antidiabetic activity of Apigenin (4, 5, 7 – Trihydroxy Flavone) from leaves of *Stachytarphetajamaicensis* (L) Vahl (Verbenaceae)

ABSTRACT

Background: Apigenin is a polyphenolic compound that belongs to the class of flavonoids and is considered highly therapeutic.

Aim: To evaluate the antidiabetic activity of apigenin isolated from dichloromethane:methanol extract of leaves of *Stachytarphetajamaicensis* (L) Vahl (Verbenaceae) in alloxan-induced diabetic rats.

Method: Cold maceration was used to extract the *S.jamaicensis* powdered leaves with dichloromethane:methanol (1:1). Hexane, dichloromethane, and aqueous methanol fractions of the extract were obtained through fractionation. To isolate the bioactive molecule, the dichloromethane fraction (DCMF) was then subjected to column chromatography and eluted with several solvent mixtures in ascending polarity order. With the aid of data from FTIR, UV, GC-MS, ¹HNMR (400MHz), and ¹³CNMR (101MHz), the structure of the isolated compound was identified. The Phytochemical analysis of the isolate and acute toxicity study was done following standard procedures. The anti-diabetic potential of the isolate was assessed by determining fasting blood glucose levels on alloxan-induced rats at the dose of 25 and 50 mg/kg body weight.

Results: The compound was identified as apigenin (4, 5, 7 – trihydroxy flavones) and showed significant ($p < 0.05$) reduction of 79.56 and 81.74 % in fasting blood glucose levels at the dose of 25 and 50 mg/kg respectively when compared with the standard drug (glibenclamide 83.40 %). The isolate's phytochemical analysis revealed flavonoids and the LD50 test demonstrates that apigenin was not harmful.

Conclusion: The findings show that the apigenin from *Stachytarphetajamaicensis* (L) Vahl has potent anti-diabetic properties.

Keywords: *Stachytarphetajamaicensis*, apigenin, antidiabetic, phytochemical analysis

Introduction

The metabolic disorder diabetes mellitus (DM), which has various aetiologies and debilitating short- and long-term repercussions, is severe, chronic, and complicated [1]. Diabetes is characterized by chronic hyperglycemia with modifications in macromolecule metabolism brought on by deficits in insulin production, action, or both. Diabetes results in long-term impairment, dysfunction, and failure of several organ systems, including the heart, blood vessels, eyes, kidneys, and nerves [2]. The overall frequency of diabetes in the adult population has increased from 4.7% to 8.5% since 1980. Furthermore, it has been found that diabetes prevalence has been increasing over the past three decades, but more swiftly in low- and middle-income countries than in high-income ones. Despite substantial research, diabetes

incidence and prevalence have risen globally, adding to the burden on developing tropical countries [3, 4]. Demographic predictions show that by 2030, developing countries will have 82 million more people over the age of 64 living with diabetes than industrialized ones (48 million). The three regions expected to see the highest relative increases are India, the Middle East Crescent, and sub-Saharan Africa [5]. Despite the existence of anti-diabetic drugs, medicinal herbs are usually successful in treating diabetes. Herbal remedies and plant components with little toxicity and no side effects are significant therapeutic possibilities for the treatment of diabetes around the world [6]. Most research has demonstrated the benefits of medicinal herbs with hypoglycemic properties in the management of diabetes.

Natural chemicals, especially those of plant origin, are a major source for locating promising lead candidates in the upcoming drug development programs [7-9]. Due to their ease of accessibility, low cost, and lack of side effects, plant-based remedies dominate the market among all readily available medications, particularly in rural areas [10] [27]. A rich source of -bioactive chemicals, which have powerful pharmacological advantages without any undesirable side effects, is also provided by a range of plants [11–15]. Throughout history, a lot of the drugs that are currently available on the market have been made either directly or indirectly from plants [11, 12]. Long regarded as the main source of potent anti-diabetic drugs, several botanicals. Diabetes is treated with medicinal plants, especially in developing countries where the high expense of conventional drugs is a burden on the population. Today, it is recommended to use medicinal plants to treat conditions like diabetes [16] because they include a number of phytoconstituents that may have anti-diabetic characteristics, including flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides.

Stachytarpheta jamaicensis (L.) Vahl is a member of the family of Verbenaceae. The tropical regions of America, as well as the subtropical forests of Asia and Africa, are home to the majority of this plant's habitats [18]. The attractive herb *S. jamaicensis* has blooms. This invasive herbaceous plant grows from 60 to 120 cm tall. Smooth and dark green in color, the stem of this plant gets woody close to the base. It is one of those frequently used plants because of its wide range of therapeutic benefits, including its anti-inflammatory [19], antidiabetic [20], hypotensive [21], antihelminthic [22], diuretic, laxative, wound healing [23], lactagogue, purgative, sedative, spasmogenic, vasodilator, vulnerary, and vermifuge properties [24–26].

S. jamaicensis has also been shown to contain a number of phytochemical elements, which are what give this plant its potent medical properties. Researchers are particularly interested in the phytochemicals present in the phenolic components of *S. jamaicensis*, which include flavonoids, tannins [27], and saponins [24], due to their therapeutic properties. These chemicals ultimately result in a variety of medicinal properties. This plant's roots stem, and leaves are frequently used in traditional medicine to cure a variety of illnesses. The objective of the present investigation was to assess the anti-diabetic potential of apigenin, a flavonoid present in the leaf tissue of *S. jamaicensis*.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Material

Fresh leaves of *Stachytarpheta jamaicensis* (L.) Vahl was collected in June from Orba, Udenu LGA, Enugu State, Nigeria, and identified by Mr. A.O Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria. The voucher specimen (UNN/PCG/14/022) was deposited in the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The leaves were air-dried under shade for three weeks. The dried leaves were then ground into powder using a grinding machine.

Animals

The animals were housed in cages under laboratory conditions at $22 \pm 2^\circ\text{C}$ and 60–65% relative humidity with a normal 12-h light and dark cycle.

Extraction and isolation of apigenin

The powdered leaves (1 kg) were thoroughly macerated for 72 hours with 5 l of a 1:1 mixture of dichloromethane and methanol. It was filtered, and a rotary evaporator was used to concentrate the filtrate in a vacuum. The resulting dichloromethane methanol extract was suspended in a 1:1 mixture of MeOH and water before being progressively partitioned into n-hexane, dichloromethane, and aqueous methanol fractions using the two solvents. Hexane:Ethylacetate gradient column chromatography is used to separate the dichloromethane phase with increasing polarity. The fractions were collected put through column chromatography, and then TLC-monitored. The solvent was mixed with similar fractions and extracted under vacuum. Melting point, IR, ^1H NMR (400MHz), ^{13}C -NMR (101MHz), and MS provided thorough descriptions of the pure chemical that was separated. The India Institute of Integrative Medicine (iiim) in Jammu, India, used an FTIR Perkin

Elmer to record the infrared spectrum, and a Bruker Advance II 400 NMR spectrometer to record the ¹H- and ¹³C-NMR spectra while utilizing CDCl₃ as a solvent.

Test for flavonoids

A 3ml solution of 10% lead acetate was used to treat the isolated chemical. The presence of phenolic chemicals was indicated by an expansive white precipitate.

Acute toxicity study

The median lethal dosage (LD₅₀) was determined using Lorke's [27] method. The twelve mice used in this study had weights ranging from 18 to 40g. The animals were given only water for the 18 hours leading up to the research while fasting. For the animals, six treatment groups, referred to as groups "A to F," were made. All medications were administered orally. There were three animals in each of the groupings A through C. While groups B and C received 100 and 500 mg/kg of extract, respectively, Group A received 10 mg/kg of extract. Groups D, E, and F each received 1600, 2900, and 5000 mg/kg of extract, with just one mouse in each group. The animals were observed for toxicity-related signs and symptoms, including mortality, for 24 hours after treatment. The geometric mean of subsequent doses for which survival rates of 0 and 100% were reported was multiplied by the square root of the product of the lowest lethal dose and the highest non-fatal dose to arrive at the final LD₅₀.

Induction of diabetes

Twenty-four (24) white albino Wistar rats were used for the study. They were given free access to water and fed with grower's mash commercial feed. Before inducing diabetes in the rats, the basal blood glucose level was measured by taking a blood sample from the tail vein end of the animals and using an Accu-check glucometer to calculate the glucose level. Then diabetes was induced by injecting 150 mg/kg body weight of fresh prepared alloxan monohydrate intraperitoneally. After three days of induction, the animals were fed normally with food and drink, and those with a fasting blood glucose level of 200 mg/dl or more were deemed diabetic [28].

Antidiabetic effect

Twenty-four (24) healthy albino Wistar rats were used for the study. The animals were divided into four groups of six animals each. They were fasted overnight for 12 hr. At the end of the fasting period, different doses of apigenin were given to the rats intraperitoneally. Group 1 received 25 mg/kg of apigenin, Group 2 received 50 mg/kg of apigenin, Group 3

received 5 mg/kg of Glibenclamide as the positive control and Group 4 received distilled water only as negative control (diabetic control). Blood samples were withdrawn from the tail vein of each animal rat at 0, 1/2, 3, 5, 7, 9, 12, and 24h [28], and their blood glucose levels were estimated using Accu-chekActive™ glucose strips in Accu-Chek Active™ Test glucometer (USA).

Statistical analysis:

All values were expressed as mean \pm standard error of means. Statistical analyses were performed using Student's t-test. Values with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Spectroscopic data of isolated compound

Apigenin (Fig. 1, SJ2) was isolated as yellow crystalline powder (50mg) with a melting point of 345 – 350 °C and Rf value of 0.49 (10% EtOAc/Hexane).

The molecular ion peak in the EI-MS spectra was identified as 271.05g/mol (Molecular Formula C₁₅H₁₀O₅) (Fig. 2). At the following m/z, characteristic fragments were located: 271, 243, 213, 193, 179, 158, 144, and 118. Tables 1 through 4 display the quantitative compound report.

¹H NMR (400 MHz, MeOD) has given signals at δ 12.9 (s, 1H, Ar-OH), 10.40 (s, $J = 8.8$ Hz, 1H), 9.65 (d, $J = 8.8$ Hz, 1H), 7.49 (d, 1H), 7.47 (s, $J = 8.8$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 1H), 6.63 (s, 1H), 6.64 (d, $J = 2.1$ Hz, 1H), 6.07 (d, $J = 2.1$ Hz, 1H) 5.84 (s, 1H) (Tables 5).

¹³C NMR (101 MHz, MeOD) δ 182.62(C-4), 166.97(C-7), 165.23(C-2), 161.41(C-5), 158.07(C-9), 157.01(C-4¹), 128.16(C-2¹, C-6¹), 122.08(C-1¹), 115.85(C-5¹, C-3¹), 105.67 (C-3), 99.11(C-6), 94.07(C-8) (Table. 5).

IR spectra showed absorption peaks at 3276.87 cm⁻¹ (O-H stretching), a strong band at 2918.20 cm⁻¹ (aliphatic C-H stretching indicating the presence of -CH₂-and-C-H), 2851.90 cm⁻¹ (asymmetric and symmetric -C-H stretching of CH₂ group), 1649.49 (C=C absorption), 1605.06, 1586.90 and 1554.39 cm⁻¹ (indicates the presence of an aromatic ring system and 1443.27, 1352.39 cm⁻¹ (CH bending). Other absorption frequencies include 1267.95, 1242.88, and 1180.43 cm⁻¹ (C-O stretching absorption), 1030.43 cm⁻¹ (C-C stretching of cycloalkane), and 906.60, 826.97, 805.02, and 736.31 cm⁻¹ (bending frequency for cyclic

(CH₂)_n). The IR spectra at 3276.87 cm⁻¹ (Fig. 3) revealed that the nature of oxygen is a hydroxyl group.

According to the NMR data, the skeleton contained three rings, seven olefin bands, and one carbonyl carbon. In deuterated dimethylsulfoxide (DMSO), ¹H-NMR measurements were made. The proton at position 2 and position 6 due to the symmetrical structure emerged as a doublet at 7.69 ppm and proton of 3 at 6.79 ppm, upfield due to the adjacent electron-donating (hydroxyl) group (Fig. 4). The hydroxyl proton first showed at 12.9 ppm. Doublet at 8.07 ppm was produced by protons. The molecule included 15 carbons, according to the ¹³C NMR data. We were able to determine the structure of component 2 as apigenin or 4', 5, 7-trihydroxyflavone [29, 30] by comparing the spectrum data to those previously reported.

Test for Flavonoids

The spectrum findings are consistent with the flavonoid test for apigenin being positive. The vast class of polyphenolic chemicals known as flavonoids include flavones, flavanones, flavanols, isoflavones, anthocyanidins, and flavonols. Flavonoids are generally present in all foods that are derived from plants. For more than a century, flavonoids have been recognized as a significant plant product. For the first time, Rusznyak and Szent-Gyorgyi published material in 1936 discussing their biological activity [31]. Flavonoids have a wide range of pharmacological and biochemical actions including anti-inflammation, antioxidant, anti-platelet, anti-allergic, and anti-thrombotic [32-35]. According to Yamagata et al. [35] and Pinheiro *et al.* [37], apigenin has antinociceptive and antihyperglycemic effects in addition to antioxidant, anticancer, and anti-inflammatory properties. Apigenin are natural food additive and is widely employed in the pharmaceutical and food industries. According to Patel, flavonoids make up a sizable portion of the worldwide nutraceuticals business [38].

LD₅₀ test

Throughout the experiment, the mice who had been treated seemed to behave normally. At doses up to 5000 mg/kg, no hazardous effects were noticed, and none of these groups experienced any fatalities.

Antidiabetic effect of apigenin

The physiological and biochemical anomalies of the diabetic condition have received a considerable lot of attention in experimental diabetes models in animals. Alloxan is often used as a diabetogenic agent in study animals. Additionally, using an alloxan-induced rat

model, the antidiabetic effects of several plant constituents have been studied [39]. Alloxan induces diabetes mellitus by destroying the insulin-producing beta cells of the islets of Langerhans in the pancreas. Alloxan's diabetogenic effects are also brought on by the cytotoxicity of reactive oxygen species overproduction in pancreatic beta cells, which decreases insulin synthesis and release [40].

In the current study, an increase in blood glucose levels in diabetic rats induced with alloxan compared to control rats served as evidence of this influence. Epidemiological research and clinical trials have shown that hyperglycemia is the primary cause of diabetes complications [41]. The constant lowering of hyperglycemia or effective glucose control is the key to treating or curing the illness. The effects of apigenin on the fasting blood levels and the percentage reduction in blood glucose levels in diabetic rats are shown in Tables 6 and 7. According to the results, the 25mg/kg dose of apigenin had a stronger effect at 1 and 3 h after administration, compared to the 50mg/kg dose, which demonstrates a statistically significant effect, and a significant ($p > 0.05$) reduction in blood glucose was seen at both of the provided doses.

Apigenin had a more positive overall effect than Glibenclamide, the positive control (regular medication). However, the fasting blood sugar level reduced substantially and significantly ($p > 0.05$) during the course of all the hours in diabetic rats given 50 mg/kg of the extract. On the 24th hour, the blood glucose levels in the 25 and 50 mg/kg groups fell by 79.56 and 81.74%, respectively, compared to an 83.40% drop in the traditional treatment group. Those in the diabetic control group had higher blood sugar levels. These results demonstrate that apigenin dramatically lowers blood sugar levels in mice with alloxan-induced diabetes.

Conclusion

The principal pharmacological action of *S. jamaicensis* can be investigated in the treatment of illnesses in order to produce successful traditional pharmaceuticals, as the pharmaceutical industry looks forward to producing exclusive drugs from natural sources. A thorough research and development effort should be conducted with the aim of improving the economic and therapeutic usage of new products before they are developed and introduced to the public.

Figure 1: Apigenin (SJ 2), C₁₅H₁₀O₅, Mol. Wt: 270.24g/mol

Table 1: Qualitative compound report

Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB Formula
Cpd SJI: C15 H10 O5	0.3	270.0524	C15H10 O5	C15H10 O5	1.49	C15H10 O5

Table 2: Alogrithm and mass to charge ratio

Compound Label	m/z	RT	Algorithm	Mass
Cpd SJI: C15 H10 O5	271.0597	0.3	Find by molecular feature	270.0524

Table 3: Peak list of the mass spectrum

m/z	Z	Abundance	Formula	Ion
271.0597	1	114738.79	C15H1105	(M+H)+
272.0629	1	19651.59	C15H1105	(M+H)+
273.0658	1	3605.04	C15H1105	(M+H)+
274.0688	1	386.51	C15H1105	(M+H)+

Table 4: Predicted isotope match

Isotope	m/z	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
1	271.0597	271.0601	1.44	100	100	82.91	83.98
2	272.0629	272.0635	2.07	17.13	16.54	14.20	13.89
3	273.0658	273.0658	-0.05	3.14	2.31	2.61	1.94
4	274.0688	274.0684	-1.39	0.34	0.23	0.28	0.19

Table 5: ¹HNMR (δ_H in ppm, 400MHz) and ¹³CNMR (δ_C in ppm, 101MHz) chemical shift values for Apigenin (SJ 2)

Carbon atom	¹³ C NMR Experimental	¹ H NMR Experimental
C-1	-	-
C-2	165.23	-
C-3	105.67	6.79 (d, <i>J</i> = 8.8 Hz, 1H)
C-4	182.62	-
C-5	161.41	12.9 (s, 1H, Ar-OH)
C-6	99.11	5.84 (s, 1H)
C-7	166.97	10.40 (s, <i>J</i> = 8.8 Hz, 1H),
C-8	94.07	6.07 (d, <i>J</i> = 2.1 Hz, 1H)
C-9	158.07	-
C-1 ¹	122.08	-
C-2 ¹	128.16	7.49 (d, 1H),
C-3 ¹	115.85	6.63 (s, 1H)
C-4 ¹	157.01	9.65 (d, <i>J</i> = 8.8 Hz, 1H),
C-5 ¹	115.85	6.64 (d, <i>J</i> = 2.1 Hz, 1H)
C-6 ¹	128.16	7.47 (s, <i>J</i> = 8.8 Hz, 1H),

MFE MS Spectrum

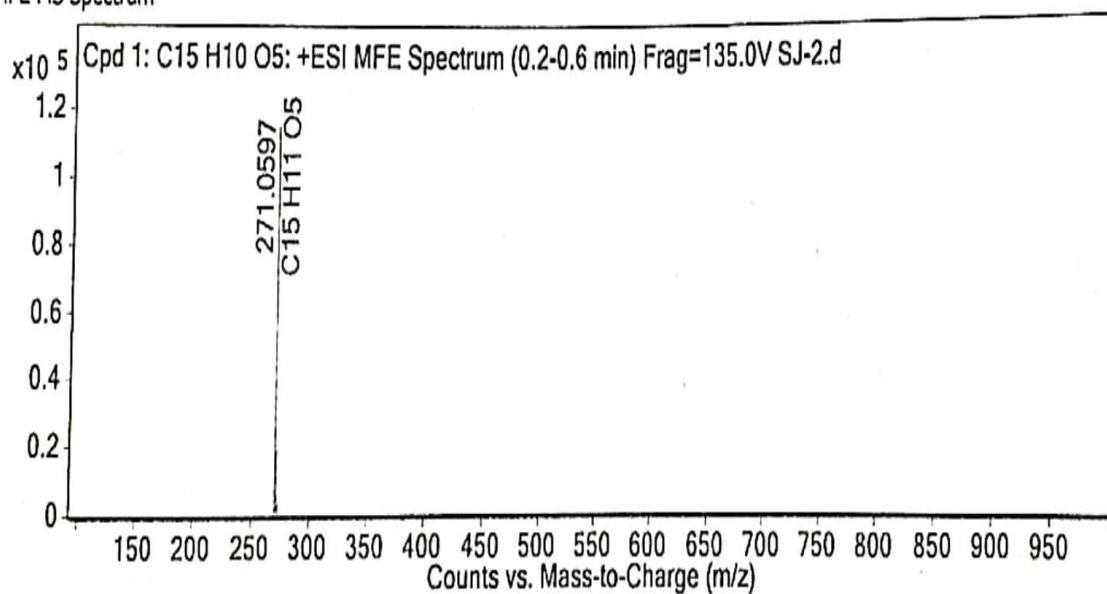


Figure 2: Mass spectrum of Apigenin (SJ 2)

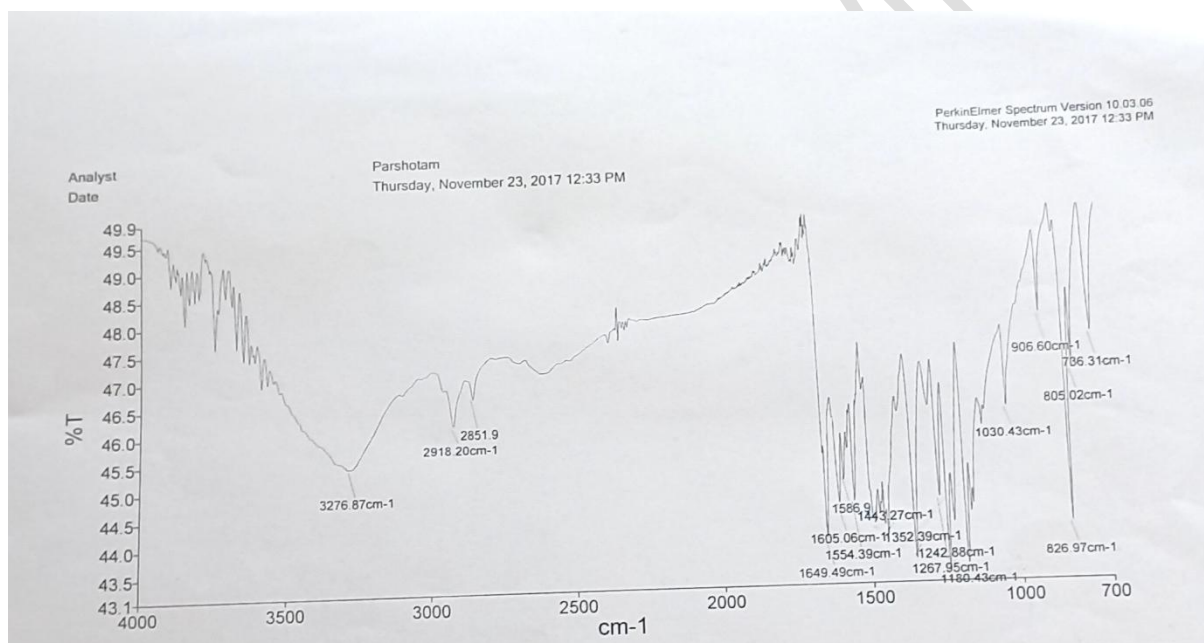


Figure 3: The IR Spectrum of Apigenin (4', 5, 7-trihydroxyflavone)



Figure 4: The ¹H-NMR Spectrum of Apigenin (4', 5, 7-trihydroxyflavone)

Table 6: Results of antidiabetic effect of apigenin on the fasting blood glucose level of alloxan induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose (mg/dl)							Percentage reduction (%)
		0h	1h	3h	6h	9h	12h	24h	
Apigenin	25	332.60 ±59.85	170.67 ±29.16*	130.67 ±30.75*	154.00 ±56.11*	86.67 ±8.57*	73.00 ±8.08*	68.00 ±7.02*	79.56
Apigenin	50	429.00 ±69.08	127.67 ±41.45*	94.33 ±34.45*	80.00 ±11.85*	81.67 ±5.04*	78.33 ±2.85*	79.33 ±6.69*	81.51
Glibenclamide	5	433.67 ±49.16	178.67± 29.46*	152.00 ±31.43*	97.33 ±5.67*	74.67 ±4.41*	73.67 ± 3.18*	72.00 ±6.43*	83.40
Negative control		477.67 ±38.40	457.67± 83.48	435.67 ±83.14	502.33 ±49.08	526.00 ±59.18	577.33 ±22.67	600.00 ±0.00	-

Values are the mean ± SEM (n=6), * p<0.05 Vs negative control

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