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HPLC Profile of Phenolic Contents, Antioxidant and Antidiabetic Activities of Methanolic Extract of the Leaves of *Sarcocephalus latifolius* (Bruce, Smith) Grown in North Central Geopolitical Zone, Nigeria**A.T. Ajiboye^{a,b*}, O.T. Asekun^b and O.B. Familoni^b**^a Department of Chemical, Geological and Physical Sciences, Chemistry Unit, Kwara State University, Malete, Nigeria.^b Department of Chemistry, University of Lagos, Faculty of Sciences, Akoka, Nigeria.Received: 15th Sep. 2019;Accepted: 7th Dec. 2020

Abstract: Preliminary phytochemical screening of the *n*-butanol fraction of the methanol leaf extract of *Sarcocephalus latifolius* was carried out; its phenolic contents were quantified by HPLC. The *in vitro* antihyperglycemic and antioxidant activities of the fraction were evaluated. The results of the present study indicated that the leaves' extract contained phytochemicals, like phenols, flavonoids, alkaloids and glycosides. Phenolic compounds, such as gallic acid, catechin, ferulic acid, *p*-coumaric acid, apigenin-7-glucose, kaempferol, quercetin and amentoflavone, were quantified by HPLC. Moreover, the fraction of leaves' extract of *S. latifolius* demonstrated significant antioxidant and antihyperglycemic activities. The presence of these phytochemicals, especially phenols and flavonoids, in the leaves of *S. latifolius* therefore justifies its medicinal usefulness, as this plant has the potential to protect oxidative stress-induced ailments.

Keywords: *Sarcocephalus latifolius*, Phenolic compounds, Antioxidant, Antihyperglycemic, HPLC.

Introduction

Sarcocephalus latifolius (Synonym-*Nauclea latifolia*) is a member of the Rubiaceae family that grows in many tropical and subtropical regions of Africa and Asia and is used for medicinal purposes in folk medicine^[1]. It is commonly found in Senegal, Cameroon and Nigeria and as far as Sudan, tropical and Southern Africa. Different parts of this plant are commonly prescribed traditionally as a remedy for diabetes mellitus, gastrointestinal tract disorders^[2], sleeping sickness^[3], prolong menstrual flow^[4], hypertension^[5] and in the treatment of malaria^[5-7]. The presence of various phytochemicals in this plant, such as alkaloids, saponins, terpenoids, anthraquinone and tannins, has been reported^[8,9]. Alkaloids, such as strictosamide, naucleamide (A-E)^[8], latifoliamide (1-5)^[9] and 19-O-ethylangustoline^[10], have been re-

ported to be present in the stem bark of this plant. Two new indole alkaloids; 21-O-ethylstrictosamide aglycone and 21-O-methylstrictosamide aglycone, are present in the root of *S. latifolius*^[10]. Recently, two bisdemolides tripterpenoid glycosides (quinovic acid 3-O- α -L-rhamnopyranosyl 28;- β -D-glucopyranosyl ester and quinovic acid 3-O- β -D-fucopyranosyl-28- β -D-glucopyranosyl ester) and a monodesmoside triterpenoid glycoside (quinovic acid 3-O- β -D-glucopyranoside) were isolated for the first time from the ethanol root extract of *S. latifolius*^[11].

Polyphenolic contents of the aqueous extracts of the leaves and fruits of *N. latifolia* (*S. latifolius*) were determined using the Folin-Ciocalteu reagent by Ademola *et al.*^[12]. It was reported that the aqueous extract of the leaves contained a higher level of total polyphenols, flavanol and flavonol than the extract of the

*Corresponding Author: A.T. Ajiboye

Email: abdulfatai.ajiboye@kwasu.edu.ng

fruits. More so, phenolic compounds, such as chlorogenic acid, catechin, cynaroside, tangeritin, rutin/hesperidin, and hyperoside, were determined in the aqueous leaves' extract, while syringic acid, nobiletin, tangeritin, catechin, chrysoeriol and isorhamnetin were found in the fruits' extract using LC-MS^[12]. GC-MS analysis of *n*-butanol and ethylacetate fractions of the methanol leaf extract of *S. latifolius* revealed the presence of quinic acid in both fractions. The amount of quinic acid was higher in the butanol fraction than in the ethylacetate fraction^[13].

Literature survey revealed that the application of GC-MS for qualitative and quantitative determination of phenolic compounds has been restricted by the limited volatility of many phenolic compounds, as well as by the necessity to derivatize the samples prior to analysis. Therefore, reverse-phase (RP) HPLC is currently the most popular and reliable technique for the analysis of phenols, since it requires no sample derivatization^[14,15].

In this study, the butanol fraction of the methanol extract of *S. latifolius* was investigated using HPLC, for the first time to the best of our knowledge, for its phenolic contents and its antioxidant and antihyperglycemic potentials.

Materials and Methods

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), α -amylase, α -glucosidase, hydrogen peroxide, acarbose, ascorbic acid (Vitamin C), methanol and *n*-butanol were obtained from Sigma-Aldrich (Germany).

The leaves of *S. latifolius* were collected from Kwara State University environment, Malete, Moro LGA, Kwara State, Nigeria. The plant was identified and authenticated by Mr. Odewo Samuel of Forestry Reserve Institute of Nigeria (FRIN) Jericho, Ibadan, Oyo State, Nigeria, where its voucher number (FHI 112260) was deposited. The leaves were washed under running tap water, cut into smaller pieces and dried at room temperature for 7 days. The dried leaves were pulverized into powdery form and kept in a polythene bag until further use. A sample of 200 g of the air-dried pulverized leaves was macerated in methanol (95%) for 24 hours. The extract was filtered, and the residue was re-extracted thrice in the same manner with a fresh batch of solvent. The collected filtrates were pooled and concentrated over reduced pressure by rotary

evaporator at 40 °C to afford 15.5 g (7.8%) of total crude extract (dark green solid).

A 2.0 g-sample of the crude methanol extract was dissolved in distilled water (10 ml) and stirred in a 100-ml round bottom flask for 2 hours at room temperature. The water-soluble fraction was partitioned with *n*-butanol (3×25 ml). The butanol fractions were pooled and concentrated to afford a reddish-brown syrup-like substance coded BFS (butanol fraction of *S. latifolius*). TLC analysis of BFS was conducted on a normal phase silica gel-coated plate using the solvent system CH₃Cl/EtOAc/MeOH/H₂O (4:6:4:1). The plate was air-dried and visualized by UV light at 254 nm, after heating following spraying with vanillin-H₂SO₄ reagent. Ferric chloride reaction test was also conducted on the developed chromatogram by spray with ferric chloride solution.

The phenolic contents of the sample were analyzed by HPLC (Agilent 1100 series with UV-Detector G1314A (Japan), a Zorbax eclipse Auto-sampler and a 5-micro column compartment (Germany), Rheodyne auto injector USA (20 μ L loop volume), Degasser G1719A (Japan), coupled with fluorescent and UV detector). The chromatographic separation was performed on automated HPLC system driven by ChemStation software using a hypersil 100-5 (Macherey-Nagel) silica gel-coated column (150 × 4.6 mm).

The test samples (10 mg/ml) and reference compounds (5, 10, 25, 50, 100, 175, 250 and 500 μ g/ml) were filtered with a syringe filter (0.45- μ m pore size). Injection volume was 20 μ l and oven temperature was 30 °C. Flow rate was 1 ml/min and run time was 60 min. Aqueous methanol (10%v/v, 1 ml) served as a suitable solvent medium for both the standard and test samples. The mobile phases selected for method validation and for the determination of the phenolic compounds were methanol, acetonitrile and purified water containing 0.1% formic acid. Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The samples were monitored with UV detector at 280 nm. Each of the reference compounds and test samples was run in triplicate at the various concentrations for precision of the method^[16] to obtain the retention time. Reference and test samples were run under the same conditions. Identification of phenolic compounds was based on co-chromatography using standards of the phenolic compounds, including gallic acid, catechin, ferrulic acid, *p*-

coumaric acid, apigen-7-glucoside, quercetin, keamferol and amentoflavone.

Preliminary Phytochemical Screening

Phytochemical screening was conducted on the BFS according to standard methods^[17,18] to ascertain the presence of phytochemicals such as phenols, flavonoids, steroids, alkaloids, terpenoids, saponins and tannins.

DPPH Radical Scavenging Activity

Free radical scavenging activity of BFS was evaluated using DPPH assay according to the method described by Khatua et al.^[19] with slight modifications. 0.004 g DPPH reagent was dissolved in 100 ml methanol. In 96-well plates, 50 µl of sample solution (concentration ranges from 10 to 150 µg/ml) and 150 µl DPPH solution were added. The plate was incubated for 30 min at room temperature in the dark and finally, absorbance was recorded at 595 nm wavelength. Ascorbic acid at different concentrations ranging from 10 to 150 µg/ml was used as a standard. Methanol was used as a negative control. Samples, standards and control were run in triplicates. The degree of scavenging was calculated using the following equation, where *A* stands for absorbance:

$$\% \text{scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

ABTS Radical Scavenging Assay

ABTS assay was carried out according to the method described by Khatua et al.^[19] with modifications. ABTS radicals were prepared freshly according to the original method. After 12-16 h, the solution was diluted to an absorbance of 0.9 ± 0.02 at 745 nm. In 96-well plates, 50 µl of sample solution (concentration ranges from 10 to 150 µg/ml) and 150 µl of ABTS radical solution were added. The plate was shaken for 10 seconds at medium speed and absorbance was measured at 745 nm following incubation in the dark for 5 min. Ascorbic acid at different concentrations ranging from 10 to 150 µg/ml was used as a standard. Methanol was used as a negative control. Samples, standards and control were run in triplicates. The degree of scavenging was calculated by the equation shown above for scavenging activity.

Hydrogen Peroxide Scavenging Activity

The assay was performed according to the method described by Ruch et al.^[20] with modifications. 100 µl of hydrogen peroxide (40 mM) prepared in phosphate buffer (pH 7.4) was added to 100 µl of extract at a concentration of 100 µg/ml in a quartz microplate and mixed. The initial absorbance was recorded at 230 nm and the mixture was incubated for 10 min. The absorbance was read again at 230 nm after 10 min. Ascorbic acid at a concentration of 100 µg/ml was used as a standard. Phosphate buffer was used as a negative control. Samples, standards and control were run in triplicates. The degree of scavenging was calculated by the equation shown above for scavenging activity.

In vitro α-amylase Inhibitory Activity

α-amylase inhibitory activity of BFS was carried out according to the standard method described by Ademiluyi and Obboh^[21] with minor modifications. In a 96-well plate, a reaction mixture containing 50 µl of phosphate buffer (100 mM, pH = 6.8), 10 µl of α-amylase (2 U/ml) and 20 µl of varied concentrations of the butanol fraction (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) was pre-incubated at 37 °C for 20 min. Then, 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37 °C for 30 min; 100 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was then measured at 540 nm using Multiplate Reader (Multiska Thermo Scientific, version 1.00.40). Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. A test without BFS substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

$$\% \text{inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

In vitro α-glucosidase Inhibitory Activity

α-glucosidase inhibitory activity of BFS was carried out according to the standard method described by Ademiluyi and Obboh^[21] with minor modification. In a 96-well plate, reaction mixture containing 50 µl of phosphate buffer (100 mM, pH = 6.8), 10 µl of α-glucosidase (1 U/ml) and

20 µl of varied concentrations of BFS (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) was pre-incubated at 37 °C for 15 min. Then, 20 µl of P-NPG (5 mM) was added as a substrate and incubated further at 37 °C for 20 min. The reaction was stopped by adding 50 µl of Na₂CO₃ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. A test without BFS substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, as given by the above equation for %inhibition.

Results and Discussion

The results of the phytochemical investigation of BFS (Table 1) showed that *S. latifolius* is rich in secondary metabolites such as, phenols, flavonoids, glycosides and alkaloids. This is contrary to the findings of Edewor who reported the presence of steroids and saponins and the absence of alkaloids from the butanol fraction of the methanol extract of *S. latifolius* obtained

from Omu Aran, Kwara South District of Kwara State, Nigeria^[13]. The difference in the distribution of these phytochemicals in this fraction could be due to some factors, such as time of collection, site or location of plant and nutrients' composition of the soil.

The presence of these phytochemicals in plants had been reported to elicit important biological and pharmacological activities^[22]. Phenolic and flavonoids in particular elicit diverse biological activities, such as antidiabetic^[23], antiviral^[24], antioxidant^[25], antiulcer and anti-inflammatory^[26], cytotoxic and antitumor^[27]. Phenols have been reported to act as α -glucosidase and α -amylase inhibitors, which could be a good approach to control post-prandial hyperglycemia and provide effective benefits without side effects^[28]. Alkaloids have been reported for their hypoglycemic activity by inhibiting α -glucosidase and decreasing the transport through the intestinal epithelium^[29]. The occurrence of these phytochemicals in the leaves of *S. latifolius* could be responsible for its biological activities.

Table 1. Phytochemical constituents identified in BFS.

Phytochemical constituents	Specific tests	Result
Phenol	Ferric chloride	++
Flavonoids	Shinoda test	++
	Alkaline reagent test	++
	Acidic reagent test	++
Alkaloids	Dragendoff's test	+
	Meyer's test	-
	Hager's test	-
	Wagner's test	+
Steroids	Liebermann-burchard's test	-
Terpenoids	Salkowski's test	-
Saponin	Frothing's test	-

++ present in large amount, + present in trace amount, - absent

The phenolic and flavonoid contents (Table 2) of the butanol fraction of the methanolic leaf extract of *S. latifolius* were identified and quantified for the first time using HPLC. Figure 1 represents two HPLC chromatograms of the fraction, indicating the presence of various phenolic compounds. Comparison with reference substances suggests the presence of gallic acid,

ferrulic acid and *p*-coumaric acid, in addition to flavonoids, such as catechin, apigenin-7-glucose, kaemferol, quercetin and amento-flavone. The structures of the identified compounds are shown in Scheme 1. Quantification was based on peak area and the determined concentrations of these compounds (Table 2) were extrapolated from a standard curve obtained by plotting the peak area

against various concentrations of the standards. The chromatograms also revealed the presence of three compounds which were not identified in this study, because they were not included among the standard used. Further studies are suggested to identify most of the peaks observed in the chromatograms using LC-MS. The *p*-

coumaric acid (13.09 ppm), Kaemferol (12.80 ppm) and amentoflavone (9.47 ppm) were respectively found to be the most abundant compounds, while catechin (4.13 ppm), apigen-7-glucoside (2.02 ppm), quercetin (1.89 ppm) and gallic acid (0.38) were detected in lower concentrations in the leaves of *S. latifolius*.

Table 2. Phenolic compounds and their concentrations in BFS as determined by HPLC.

Phenolic Compounds	Ret. Time min	Area mAU×S	Area%	Concentration (ppm)
Garlic acid	6.1	10.9	0.4	0.38
Catechin	19.7	53.4	2.0	4.13
Ferrulic acid	21.0	11.1	0.4	1.68
<i>p</i> -coumaric acid	25.3	651.9	24.8	13.09
Apigen-7-glucoside	37.1	43.4	1.6	2.02
Qurcetin	39.9	45.3	1.7	1.89
Kaemferol	42.0	84.4	3.2	12.80
Amentoflavone	42.2	374.1	14.2	9.47

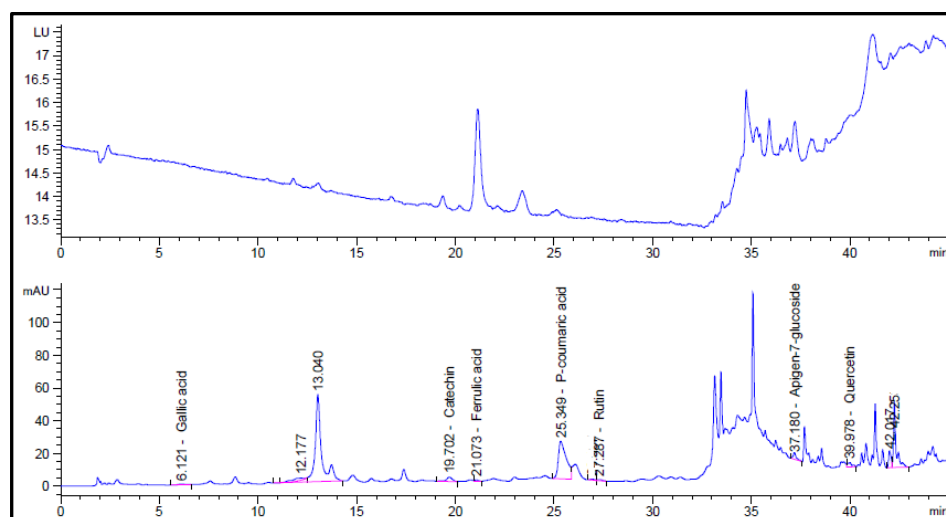
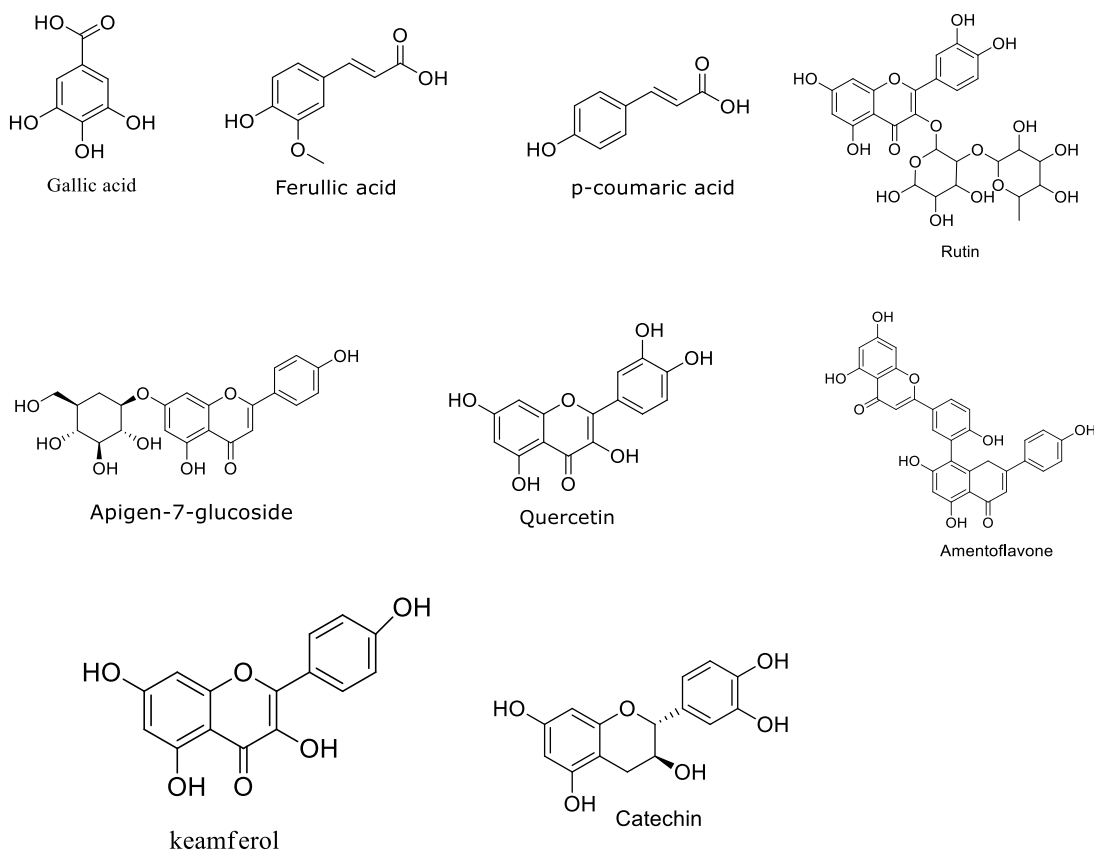


Figure 1. HPLC chromatogram of BFS with fluorescence detector (up) and VWD (down).

The result of antioxidant assay (Table 3) demonstrated that butanol fraction of methanol leaf extract of *S. latifolius* had significant inhibitory potential against DPPH, ABTS and hydrogen peroxide. The IC_{50} value of BFS ($IC_{50} = 0.790 \pm 0.001 \mu\text{g/mL}$) is very similar to that of vitamin C ($IC_{50} = 0.7110 \pm 0.002 \mu\text{g/mL}$), which is in close agreement with the findings of Ademola et al., who investigated the antioxidant activity of hot water extracts of fruit and leaves of *Nauclea latifolia* (syn. *S. latifolius*) against DPPH and found that the extract exhibited similar antioxidant effect compared to the

standard drug (Vitamin C)^[12]. Furthermore, BFS significantly demonstrated high antioxidant potentials against ABTS and hydrogen peroxide than the standard drug (vitamin C). This activity is reported for the first time, to the best of our knowledge, in this study. The strong antioxidant activity of the butanol fraction of the leaf extract of *S. latifolius* could be traced to its high phenolic and flavonoids contents and this suggests that the leaf can be considered as a viable source of natural antioxidant and might be exploited for nutraceutical use.



Scheme 1. Structures of phenolic compounds present in the leaf of *S. latifolius*, identified and quantified by HPLC.

The oxidative stress due to the formation of free radicals with glucose oxidation and the subsequent oxidative degradation of glycosylated proteins can cause diabetic complications^[30]. Therefore, the use of antioxidants along with anti-diabetic drugs is frequently recommended to avoid such complications^[31]. The results of enzyme inhibitory assays (Table 4) revealed that BFS possessed significant inhibitory potential in a concentration- dependent manner. It showed a very impressive antihyperglycemic potential against α -amylase and α -glucosidase in comparison with the standard drug (acarbose).

One of the main therapeutic approaches to managing diabetes mellitus is to retard the absorption of glucose via inhibition of digestive enzymes in the digestive organs, such as α -

glucosidase^[32,33] and α -amylase^[34]. α -glucosidase is a membrane-bound enzyme located at the epithelium of the small intestine that catalyzes the cleavage of glucose from disaccharides to monosaccharides^[35]. Inhibitors of α -glucosidase or α -amylase are used to control the blood sugar levels for type 2 diabetes mellitus. Usually, α -glucosidase inhibitors are consumed with meals, as they act to decelerate the breakdown of complex sugars into glucose, resulting in a delay in glucose absorption which lowers postprandial blood sugar levels^[36]. This study demonstrated that BFS has lowest IC_{50} value similar to the standard drug (acarbose). Thus, the results of the present study support the potentials of the leaf of this plant as a source of antidiabetic agent.

Table 3. Free radical scavenging activity of the extract.

SAMPLE	DPPH (IC_{50} μ g/ml)	ABTS (IC_{50} μ g/ml)	Hydrogen peroxide (IC_{50} μ g/ml)
BFS	0.790 $\pm$ 0.001	0.0391 $\pm$ 0.010	2.302 $\pm$ 0.002
VITAMIN C	0.7110 $\pm$ 0.002	0.088 $\pm$ 0.001	3.811 $\pm$ 0.003

Table 4. Antihyperglycemic potential of BFS.

SAMPLE	α -amylase (IC ₅₀ µg/ml)	α -glucosidase (IC ₅₀ µg/ml)
BFS	0.0062 ±0.001	0.835 ± 0.010
Acarbose	0.228 ±0.001	0.867±0.010

Conclusion

In our investigation, we found that the butanol fraction of *S. latifolius* (BFS) demonstrates the presence of phenols, flavonoids, glycosides and alkaloids (Table 1). The result of HPLC analysis also confirmed the presence of different phenolics and flavonoids (Table 2). Antioxidant activities were confirmed by DPPH, ABTS and hydrogen peroxide radical scavenging methods, while those of antihyperglycemic potentials were confirmed by α -amylase and α -glucosidase assays. It could therefore be inferred that the presence of these phytochemicals, especially phenols and

flavonoids, in the leaves of *S. latifolius* therefore justifies the medicinal usefulness of this plant in protecting from free radicals or oxidative stress-induced ailments. Further efforts are underway to isolate and identify the active phenolic, flavonoids and other compounds from the leaf part of this plant.

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