Journal of Scientific and Innovative Research 2017; 6(4): 119-125
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2017; 6(4): 119-125

Received: 25-11-2017
Accepted: 31-12-2017

Ololade ZS
Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria

Ogunmola OO
a) Department of Pure and Applied Chemistry, Ladoke Akintola, University of Technology, Ogbomoso, Nigeria
b) Department of Chemistry, Emmanuel Alayande College of Education, Oyo, Nigeria

Kuyooro SE
Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria

Abiona OO
Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria

Correspondence:
Ololade ZS
Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria

Stachytarpheta jamaicensis leaf extract: Chemical composition, antioxidant, anti-arthritic, anti-inflammatory and bactericidal potentials

Ololade ZS, Ogunmola OO, Kuyooro SE, Abiona OO*

Abstract

Stachytarpheta jamaicensis is an important plant with multi therapeutic properties. Hence, this study seeks to screen the leaf extract of *S. jamaicensis* for its chemical composition and pharmacological activities in order to find possible sources for novel phytochemicals in food and pharmaceutical formulations. The chemical composition, pH, TPC, TFC, TTC, TAA, carotenoid, antioxidant, anti-arthritic, anti-inflammatory and bactericidal potential were measured using GC-MS, pH meter, Folin-Ciocalteu's, $AlCl_3$, $FeCl_3$/gelatine, 2,4-DNPH, acetone-hexane, DPPH, PTAC, BSA and agar-well diffusion methods respectively. The pH of the aqueous solution was 6.02. The GC and GC-MS analyses revealed the presence of 30 organic compounds. The most abundant components were 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (13.7%), D-arabinitol (13.5%), 2-benzylidenemalonic acid (11.9%), 1,3-cyclopentadione (8.9%), α-5-ethyl-2-furylglycine (6.8%), 4,5-dihydro-5-methoxy-4-(2,3-dimethyl-2-buten-4-yl)-2(3H)-furanone (6.4%) and 3-methyl-2H-indazol-2-ol (5.8%). The TPC, TFC, TTC, TAA, β-carotene and lycopene values were 31,882.80 ±0.00 μgmg^{-1} GAE, 29.29±0.00 μgmg^{-1} QE, 126.47 μgmg^{-1} TAE, 53.75±0.01 μgmg^{-1} AAE, 0.17 $mgg^{-1}$and 0.14 mgg^{-1}, respectively. The antioxidant IC_{50} and AAI values of the leaf extract were 5.0 μgml^{-1} and 8.0. The extract was capable of scavenging free radicals in a range between 51.30-78.99%. The PTAC value was 396.15±0.00 μgmg^{-1} AAE. The extract also gave high egg albumin and BSA anti-arthritic/anti-inflammatory values between 22-80% with IC_{50} values of 0.04 and 0.15 $mgml^{-1}$, respectively. The extract was active against all the tested bacteria with high zones of inhibition (14.0-25.0 mm). These results showed that the leaf extract of *S. jamaicensis* could be used for the formulation of active compounds with broad activities for food and pharmaceutical industries.

Keywords: Stachytarpheta jamaicensis, phytochemical, antioxidant, anti-arthritic, bactericidal, therapeutic activities.

INTRODUCTION

Plants are important source of natural products which provide protection against diseases causing damages to human cells [1, 2]. Natural products are important sources of pharmacologically active phytochemicals in drug research, discoveries and development because of their unmatched availability of chemical diversity [3-5]. The advantages of natural products from plants therapeutics potentials include good availability, local cultural aspects, individual preferences, the increasing demand for natural and organic products, and the already validated synergic properties of the secondary metabolites [6, 7]. The medicinal properties of most of the plants around us are not fully known. Plants used locally for medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [8, 9]. There is growing interest in the use of alternative medicine derived from plants throughout the world due to the development of adverse effects and microbial resistance to the chemically synthesized drugs [10-12]. Human being found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect [13, 14]. In most cases the people claim the good health benefits of natural products. However, scientific proof and clinical trials are necessary to demonstrate the effectiveness of pharmacologically active secondary metabolites in plants to prove their traditional uses [15, 16]. Scientific research reports, proof, validity and clinical trials show understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of a therapeutic agent [17-23].

Stachytarpheta jamaicensis (L.) Vahl is one of those popularly used plants, due to its multiple medicinal

virtues. It is a member of the family of *Verbenaceae*. This plant mostly grows in the tropical regions of America, as well as in the sub-tropical forests of Africa and Asia [24]. *S. jamaicensis* is a beautiful flowering herb. It is a weedy herbaceous plant that grows between 60–120 cm tall. This plant has a smooth, dark green coloured stem, which turns woody towards the base of the stem. *S. jamaicensis* normally reproduces by flowers [25, 26]. *S. jamaicensis* has been an important plant with great medicinal properties in infectious and chronic health systems [27, 28]. However, it is not appropriate for pregnant women or individuals with low blood pressure to consume the plant, since it is considered to have abortive and hypotensive effect [29-31]. To the best of our knowledge, there is no enough scientific information on the chemical composition and pharmacological properties of *S. jamaicensis* so far. Therefore, the present research was undertaken to screen the phytochemical, pH, total phenolic content, total flavonoid content, total tannin content, ascorbic acid, *β*-carotene, lycopene, antioxidant, anti-arthritic, anti-inflammatory and bactericidal potentials of the leaf extract of *S. jamaicensis* from Nigeria.

MATERIALS AND METHODS

Collection of Plant Sample and Preparation of the Extract

The leaves of the plant were collected from Benja village, Ota, Nigeria and it was authenticated as *Stachytarpheta jamaicensis* (L.) Vahl (*Verbenaceae*). Air-dried and pulverised leaves were extracted with methanol. The mixture was then left at room temperatures for 3 days, and then subjected to filtration. The percentage yield of the concentrated extract was calculated. The extract was then kept in a refrigerator until used [32].

Measurement of pH

Pulverised fresh leaves of *S. jamaicensis* were soaked in distilled water for 3 hr and then filtered. The pH values were measured in the just filtered solution using digital pH meter [33].

Gas Chromatography-Mass Spectroscopy Analysis

The leaf methanolic extract of *S. jamaicensis* was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280 °C (temperature at 60 °C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280 °C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature [34].

Determination of Total Phenolic Content (TPC)

The TPC of the leaf extract of *S. jamaicensis* was determined using Folin-Ciocalteau reagent. 1000 μgml^{-1} of the extract was mixed with 1 ml of 10% Folin-Ciocalteu reagent in distilled water and then neutralized with 4 ml of 7.5% sodium carbonate solution. The sample was maintained at room temperature for 3 hrs with periodical shaking; the absorbance at 760 nm was measured using SM 7504 UV Spectrophotometer. The index of TPC of the extract was determined as μgmg^{-1} of gallic acid equivalent (GAE) [35].

Determination of Total Flavonoid Concentration (TFC)

The TFC of the extract of *S. jamaicensis* was determined using aluminium chloride method. Briefly, 1.0 ml of the extract, 0.10 ml of 10% aluminium chloride, 0.10 ml (1.0 M) of sodium acetate and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using SM 7504 UV Spectrophotometer. The index of TFC concentration was expressed as quercetin equivalents (QE) in µg per mg of extract. All assays were carried out in triplicate [36].

Determination of Total Tannin Content (TTC)

The TTC in the sample was determined using the $FeCl_3$ and gelatine tests. 50 ml of deionized water was added to 0.1 g of sample and boiled for 30 min. After filtration 500 ml of deionized water was added to the solution. 0.5 ml aliquot was finally added to 1 ml 1% $K_3Fe(CN)_6$ and 1 ml 1% $FeCl_3$ and deionized water was added to 10 ml volume. After 5 min, the absorbance of the solution was measured using SM 7504 UV Spectrophotometer at 720 nm. Tannic acid (TA) was used as a reference and for the calibration curve; results were expressed in μgmg^{-1} of tannic acid equivalent [37].

Determination of Total Ascorbic Acid (TAA)

0.1 ml (1000 μgm^{-1}) of the extract was added to 1.0 ml 2,4-dinitrophenylhydrazine (2,4-DNPH). It was allowed to stand for 30 min. and the absorbance was read in triplicate using SM 7504 UV Spectrophotometer at 515 nm, distilled water was used as blank, while ascorbic acid was used as a reference and for the calibration curve; the result was expressed in μgmg^{-1} of ascorbic acid equivalent [38].

Determination of Carotenoid: Lycopene and *β*-Carotene Contents

200 mg of the leaves of *S. jamaicensis* were homogenized with 10 ml of acetone-hexane mixture (ratio 4:6) to determine the lycopene and *β*-carotene contents. The homogenate was centrifuged at 4000 x g for 15 min using Uniscope laboratory centrifuge Model SM 112. Automatically, two phases separated and an aliquot was taken from the upper solution (supernatant) for measurement of optical density at 663, 645, 505, and 453 nm using SM 7504 UV Spectrophotometer. The assays were carried out in triplicates, the results were mean ± SD with acetone:hexane as blank. Lycopene and β-carotene contents were calculated according to the equations:

$$\text{Lycopene} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453};$$

$$\beta\text{-Carotene} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}.$$

Lycopene and *β*-carotene were finally expressed as mgg^{-1} fw.

Where A= absorbance recorded at specific wavelengths [39].

Determination of Free Radical Scavenging and Antioxidant Activities

(i) *In vitro* 2,2′-Diphenyl-1-picryl-hydrazyl Assay

The antioxidant and free radical scavenging of the extract of *S. jamaicensis* were measured by using 2,2′-diphenyl-1-picryl-hydrazyl. Briefly, the reaction mixture (3.0 ml) consists of 2.0 ml of DPPH in

methanol (0.004%) and 1.0 ml of various concentrations of the extract. It was incubated for 30 min. in dark, and then the absorbance was measured using SM 7504 UV Spectrophotometer 517 nm. The control was prepared by DPPH and methanol in place of sample. In this assay, the positive control was ascorbic acid. The percentage of inhibition can be calculated using the formula:

$$I\% = [(A_{blank} - A_{ext})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of blank solution and A_{ext} is the absorbance of the extract. The dose-response curve was plotted and IC_{50} value for the extract and the standard were calculated [40].

Antioxidant Activity Index: The antioxidant activity index (AAI) was calculated as:

$$AAI = [\text{DPPH initial concentration}]/[IC_{50}]$$

AAI was classified as weak, when AAI < 0.5, moderate, when AAI ranged between 0.5-1.0, strong, when AAI ranged between 1.0-2.0, and very strong, when AAI > 2.0 [41].

(ii) Phosphomolybdate Total Antioxidant Capacity (PTAC) Assay

The PTAC of the extract of *S. jamaicensis* was determined with phosphomolybdenum using ascorbic acid as the standard. 1.0 ml (1000 μgml^{-1}) of the extract solution was combined with 1.0 ml of reagent (0.6 M sulphuric acid, 28 µ M sodium phosphate and 4 µM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 ^{o}C for 90 min. after the samples had cooled to room temperature, the absorbance of the solution was measured at 695 nm using SM 7504 UV Spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent was used for the sample and it was incubated under the same conditions as the rest of the samples. The total antioxidant capacity was expressed as equivalents of ascorbic acid [42].

***In-vitro* Anti-arthritic and Anti-inflammatory activities of the Extract on Inhibition of Protein Denaturation (Egg Albumin Assay):** *in vitro* anti-arthritic/anti-inflammatory activity of the extract was evaluated against protein denaturation method using fresh hen's egg albumin. About 5 ml reaction mixtures (0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) add 2 ml of test sample at 1000, 500, 250 and 125 μgml^{-1}). Distilled water with same volume (0.2 ml) was used as control. The mixtures were incubated at 37 ^{o}C in BOD incubator for about 15 min. followed by heating at 70 ^{o}C for 5 min. After cooling to the room temperature, absorbance was measured using SM 7504 UV Spectrophotometer at 660 nm using vehicle as blank. Aspirin (3000 μgml^{-1}) was used as reference drug. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t/V_c) - 1$$

Where: V_t = absorbance of test sample, V_c = absorbance of control.

The drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration [43].

***In-vitro* Anti-arthritic and Anti-inflammatory Activities of the Extract on Inhibition of Protein Denaturation (Bovine Serum Albumin Assay):** *in vitro* anti-arthritic/anti-inflammatory activity of the extract was evaluated against protein denaturation method using BSA. Test solution (0.5 ml) composed of 0.05ml of the extract at different concentrations (1000–125 μgml^{-1}) and 0.45 ml of BSA (5% aqueous solution). Test control solution (0.5 ml) consisted of 0.05 ml of distilled water and 0.45 ml of BSA (5% aqueous solution). Product control solution consisted of 0.05 ml of the extract at different concentrations (1000–125 μgml^{-1}) and 0.45 ml of distilled water. Standard solution (0.5 ml) consisted of 0.05 ml aspirin (3000 μgml^{-1}) plus 0.45 ml of BSA (5% aqueous solution). Solutions were incubated at 37 ^{o}C for 20 min. Solutions were cooled and 2.5 ml of phosphate buffer (pH 6.4) was added to all the solutions and temperature was increased progressively up to 70 ^{o}C for 5 min. Absorbance of the resultant solution was measured using SM 7504 UV Spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was determined using the following formula:

$$I\% = [(A_{ts} - A_{pc})/A_{tcs}] \times 100$$

Where: A_{ts} is the absorbance of test solution; A_{pc} is the absorbance of the product control and A_{tcs} is the absorbance of test solution. The dose-response curve was plotted and IC_{50} value for the extract was calculated [44].

***In vitro* Bactericidal Potential**

The antibacterial potentials of the extract were carried out using agar-well diffusion method against Gram-positive bacteria: (*Enterococcus faecalis*, *Micrococcus varians*, *Streptococcus agalactiae* and *Staphylococcus aureus*), Gram-negative bacteria: (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Salmonella typhimurium*). Bacteria were incubated and grown overnight at 37 °C in Nutrient agar. The cultured bacteria were adjusted to 0.5 McFarland standards, 20 ml of sterilized Nutrient agar medium was homogenized and aseptically poured into sterile Petri dishes and plates were swabbed with inocula of the test organisms, and kept for 30 min. for adsorption. A sterile cork borer of 6mm in diameter was used to make uniform wells into which were added different concentrations (1000, 500 and 250 μgml^{-1}) of the extract. The plates were allowed to stay in a refrigerator for 1 hour to allow proper diffusion of the extract solution into the medium. Synthetic antibiotic gentamicin (10µg/disc) was used as positive control. The plates were then incubated at 37 °C for 24 hrs before visual assessment of the inhibition zones. The zone of inhibition was measured to the nearest size in millimetre (mm) using standard rule. The assay was carried out in aseptic conditions in order to achieve consistency [45].

RESULTS AND DISCUSSION

The Yield of Leaves of *S. jamaicensis*

The percentage yield of the leaf methanolic extract of *S. jamaicensis* was 5%.

pH of the Leaves of *S. jamaicensis*

The pH of the distilled water leaf extract of *S. jamaicensis* was 6.02. This is within the standard limit (pH 3.40–6.10) that assures freshness for consumption of natural products [46], this showed that the leaf of the plant had weak acidic property.

Chemical Constituent of the Leaf Extract of *S. jamaicensis*

A total of 30 compounds were identified in the leaf methanolic extract of *S. jamaicensis*, accounting for 99.4% of the total extract (Table 1), and the main constituents identified were 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (13.7%), D-arabinitol (13.5%), 2-

benzylidenemalonic acid (11.9%), 1,3-cyclopentadione (8.9%), *α*-[5-ethyl-2-furyl]glycine (6.8%), 4,5-dihydro-5-methoxy-4-(2,3-dimethyl-2-buten-4-yl)-2(3H)-furanone (6.4%) and 3-methyl-2H-indazol-2-ol (5.8%). The chemical composition of leaf extract of *S. jamaicensis* investigated in this study was entirely different from what was obtained from different species of *Stachytarpheta*. Previous studies on the chemical composition of the leaves essential oil and hexane extract of *S. gesnerioides* from Brazil showed that its composition was dominated by guaiol (56.50% and 53.52%), respectively [47].

Table 1: Chemical Composition of Leaf Extract of *S. jamaicensis*

Compound	Retention Index	Percentage Composition
methylimidocarbamate	498	2.0
glyceraldehyde	913	2.0
1,3-cyclopentadione	942	8.9
1,7-octadien-3-ol	959	2.0
1,4-anhydroerythritol	965	2.0
isothujol	1097	1.4
α-undecene	1105	1.2
2-(2,3-dimethyloxiran-2-yl)pyridine	1112	2.4
α-limonene diepoxide	1128	1.4
acetylcarbamide	1129	0.4
N,N'-diaminoethane-1,2-diimine	1138	0.3
1-pyrrolidinylacetic acid	1168	2.1
2,4-dihydroxy-2,5-dimethyl-3(2H)-furanone	1173	0.7
lilac aldehyde	1197	1.4
erythritol	1229	2.0
1,1,3,3-Tetramethylbutylthiocyanate	1243	0.2
3-methyl-2H-indazol-2-ol	1248	5.8
3,4,4a,5,8,8a-hexahydro-1(2H)-naphthalenone	1255	1.4
4-vinylphenyl acetate	1263	1.7
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	13.7
5-amino-6-nitrosopyrimidine-2,4(1H,3H)-dione	1365	0.3
α-[5-ethyl-2-furyl]glycine	1466	6.8
4,5-dihydro-5-methoxy-4-(2,3-dimethyl-2-buten-4-yl)-2(3H)-furanone	1482	6.4
D-arabinitol	1491	13.5
α,*α*-diglycerol	1504	2.0
2-benzylidenemalonic acid	1790	11.9
2,2-diheptyl-1-oxohydrazine 1-oxide	1844	3.0
1,5-dinitroso-1,5-diazocane	1854	1.3
5-[oxido(oxo)hydrazono]hexahydroimidazo[4,5-d]imidazol-2(1H)-one	2037	0.3
rifamycin	5742	0.9
Percentage Total		**99.4**

Total Phenolic Content (TPC)

The TPC of the extract was 1,882.80 µgmg-1 GAE (Table 2). This might be due to the presence of low molecular mass phenolic compounds such as rifamycin, 4-vinylphenyl acetate, 2-benzylidenemalonic acid and rifamycin in the leaf extract. TPC determined in this study for this extract is higher than those reported in other *Stachytarpheta* species such as the aerial extracts of *S. caynnensis* with TPC between 1.83 to 15.33 g per 100 g[48]. The high phenolics content of the leaf methanolic extract of *S. jamaicensis* indicates high antioxidant and therapeutic potentials because the phenolics constituents can react with active oxygen radicals such as hydroxyl radical, superoxide anion radical and lipid peroxy radical [49,50].

Total Flavonoid Content (TFC)

The TFC of the leaf extract investigated in this study was 29.29 µgmg^{-1} QE (Table 2), this is comparable to the TFC of the leaf extracts of *S. gesnerioides* with TFC between 0.68 and 13.65 mg per 100 mg[47], while the aerial extracts of *S. caynnensis* had TFC between 2.69 to 6.21 g per 100 g[48]. The results obtained in this study showed that phenolic and flavonoid compounds may be the major contributors for the medicinal activities of the leaf extract. Consumption of flavonoids has protective effects in immunomodulation, cognition, and risk reduction of certain cancers, cardiovascular and skin diseases, neurodegenerative disorders, osteoporosis and obesity, as well as relief of menopausal symptoms [51-53].

Total Tannin Content (TTC)

The TTC of the extract investigated in this study was 126.47 µgmg^{-1} TAE (Table 2). This value is quantitatively higher than what was obtained for the various parts of three species of *Stachytarpheta* from Akwa, Southern Nigeria with their values ranged between 0.99-5.96[54]. Tannins are astringent plant phenolic compounds used therapeutically as antimicrobial, anti-inflammatory, antidiarrheal, haemostatic and antihemorrhoidal compounds. Natural products rich in tannins can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron resulting in a pathological increase in total body iron stores. Tannins stop infection while they continue to heal the wounds, burns and stop bleeding internally. They are also effective in protecting the kidneys. They have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhage, fatigue, skin ulcers [55, 56].

Total Ascorbic Acid (TAA)

The TAA analysis of the investigated leaf methanolic extract of *S. jamaicensis* showed the presence of high amount (53.75±0.01 µgmg^{-1} AAE) of ascorbic acid and its derivatives (Table 2). Ascorbic acid is an essential nutrient with potent antioxidant properties. High-dose ascorbic acid improved oedema and respiratory function in critically ill patients with severe burn injury, decreased organ failure and rapid healing in patients after major surgery[57]. It is effective as a protectant against a variety of toxic chemical agents including heavy metals[58]. The pharmacological importance of ascorbic acid is that it plays a cofactor role, as a reducing agent, in various enzymatic reactions. It has the potential to react with almost all other oxidized free radicals because it has a low redox potential of 280 mV. Therefore, it is used as an antioxidant and high doses of ascorbic acid can be used in the treatment of cancer [59].

Table 2: TPC, TFC, TTC and TAA of the Leaf Extract of *S. jamaicensis*

TPC	TFC	TTC	TAA
1,882.80 ±0.00	29.29±0.00	126.47±0.00	53.75±0.01
µgmg^{-1} GAE	µgmg^{-1} QE	µgmg^{-1} TAE	µgmg^{-1} AAE

Data are presented as the mean value ± S.D. of triplicate

Determination of Carotenoid: Lycopene and *β*-carotene

The carotenoid content (*β*-carotene and lycopene) of the extract was observed to be 0.17 and 0.14 mgg^{-1} respectively (Table 3). Carotenes

have been proved to possess antioxidant activity due to their abilities to quench singlet oxygen and inhibit lipid peroxidation [60, 61]. The molecular structures of lycopene and *β*-carotene are arranged in many forms which have conjugated double bonds in the chain resulting in powerful antioxidant performance [62]. Carotenoid exhibits a central role against cancers, cardiovascular diseases and HIV infection and other age related disorders [63, 64]. Consumption of natural products with lycopene and *β*-carotene has potentials in reduction in the risk of prostate cancer and modulate the cell cycle and induce apoptosis in different tumor lineages. Moreover, they play a crucial role in the control of intercellular communication through connexin expression modulation [65, 66].

Table 3: *β*-carotene and Lycopene of the Leaf Extract of *S. jamaicensis*

Carotenoid	Concentration (mgg^{-1})
β-carotene	0.17
Lycopene	0.14

Free Radical Scavenging and Antioxidant Activities

The percentage inhibitions of the extract at various concentrations (10-750 μgml^{-1}) were between 51.30-78.99%. The methanolic leaf extract of *S. jamaicensis* IC_{50} value of 5.0 μgml^{-1} which was lower than that of the reference compound ascorbic acid, which had an IC_{50} value of 9.0 μgml^{-1}. The leaf extract had a very strong AAI value of 8.0 (Table 4). The related species such as root extract of *S. caynnensis* had IC_{50} of 62.21 μgml^{-1} [67] while the aerial extracts of *S. caynnensis* gave EC_{50} between 38.60 and 288.44 μgml^{-1} [48]. Therefore, the leaf extract of *S. jamaicensis* investigated in this study had higher antioxidant than the reference compound and related species.

Phosphomolybdate Total Antioxidant Capacity (PTAC)

The PTAC of leaf extract of *S. jamaicensis* was found to be moderately high (396.15±0.00 μgmg^{-1} AAE) as shown in Table 4. The phosphor-molybdenum method is quantitative since the PTAC is express as ascorbic acid equivalents. PTAC assay measures the reduction degree of Mo (VI) to Mo (V). It is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand [68-70]. It involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature. It gives a direct estimation of reducing capacity of antioxidant. It remains intact irrespective of concentration of free metal ions. It shows uniqueness among *in vitro* antioxidant assays [71, 72]. It forms a green phosphomolybdenum complex without induction of free metal ions solution[68]. Natural antioxidants had attracted a wide range of interest across the globe in recent times because they possess multi-therapeutic activities and provide enormous scope in correcting imbalances in health, they are also very important in food and pharmaceutical industries [5, 73]. Human body has an inherent antioxidative mechanism and many of the pharmacological functions such as the anti-inflammatory, anti-mutagenic, antimicrobial, anti-carcinogenic, and anti-aging responses originate from antioxidant property [74, 75].

Table 4: Antioxidant Properties of the Leaf Extract of *S. jamaicensis*

Extract and Reference Drug	Percentage Free Radical Scavenging at Different Concentrations (μgml^{-1})					DPPH IC_{50} μgml^{-1}	AAI	PTAC μgmg^{-1} AAE
	10	100	250	500	750			
Extract	51.30	54.80	68.49	78.41	78.99	5.0	8.0	396.15±0.00

Anti-Arthritic and Anti-Inflammatory Potentials

Leaf methanolic extract of *S. jamaicensis* had significantly high (22-80%) anti-arthritic/anti-inflammatory potentials with IC_{50} 0.04 and 0.15 $mgml^{-1}$ against the denaturation of egg albumin and BSA respectively, as compared to the synthetic drugs (aspirin) (Table 5 and 6). The arthritic/anti-inflammatory activities may be due to the synergistic effects of the phytochemicals in the extract. Rheumatoid arthritis is a form of an auto-immune bone destructive disease, affects at least 1% of the population in the industrialized world with higher frequency in women [76]. Auto-immunity plays a pivotal role in both its chronicity and progression, and rheumatoid arthritis is considered as a systemic autoimmune disease [77]. Rheumatoid arthritis is a chronic, inflammatory, autoimmune disease especially in the peripheral movable joints, most particularly hand, knee and shoulder joints. In complex cases of rheumatoid arthritis, the synovial inflammation leads to particular cartilage damage, bone erosion, and subsequent change in joint integrity [78]. The symptoms of active rheumatoid arthritis include pain, swelling, morning stiffness, warmth, redness, and limits in the functions of the joints [79]. The systemic ramifications of the disease, apart from morbidity and mortality, include cardiopathy, nephropathy, vasculopathy and pulmonary and cutaneous disorders [80, 81].

Table 5: Egg Albumin Anti-Arthritic/Anti-Inflammatory Activity of the Leaf Extract of *S. jamaicensis* and Reference Drug

Conc. μgml^{-1}	% Inhibition	IC_{50} $mgml^{-1}$	% Inhibition of Aspirin 3 $mgml^{-1}$
1000	80	0.04	90
500	62		
250	62		
125	60		

Table 6: Bovine Serum Albumin Anti-Arthritic/Anti-Inflammatory Activity of the Leaf Extract of *S. jamaicensis* and Reference Drug

Conc. μgml^{-1}	% Inhibition	IC_{50} $mgml^{-1}$	% Inhibition of Aspirin 3 $mgml^{-1}$
1000	56	0.15	40
250	44		
125	22		

Antibacterial Potentials

The antibacterial screening of the leaf extract of *S. jamaicensis* gave wide range of zones of inhibition. The zones of inhibition of the leaf extract of *S. jamaicensis* (14.0–25.0 mm) extract had high bactericidal activities from sensitive to ultra-sensitive as compared to synthetic antibiotic (gentamicin) (Table 7). The antibacterial properties of the extract investigated in this study had a comparable activities as the extract of a related species such as the leaf and stem ethanolic extracts of *S. caynnensis* from Nigeria which showed moderate inhibitions against *P. mirabilis*, *K. pneumonia*, *P. aeruginosa* and *E. coli* between 7.0–14.0 mm but resistant to *S. aureus* [82]. Moreover, the root ethanolic extract of *S. caynnensis* from Brazil which had moderate inhibitions against *B. subtilis*, *S. saprophyticus*, *S. epidermidis*, *S. aureus*, and *S. pyogenes* between 10.0–25.0 mm[67]. In this study, the extract showed promising activities against the tested bacteria. This is due to the presence of phenolic compounds such as rifamycin, an antibiotic used to prevent and treat tuberculosis and other bacteria infections [83, 84]. Likewise, synergistic actions of other phytochemicals in the extract are also accountable for bactericidal activities against the growth of the bacteria [85, 86]. This therefore, supports the local medicinal use of the plant and suggests that it can combat multi-drug resistant pathogens that have been causing serious harm to man and animals. Infectious diseases are the leading cause for morbidity and mortality in developing and under developed countries where they are resistance to antimicrobial agents is a common scenario [87, 88]. Multi-drug resistance has been displayed not only by the pathogenic microbes but also by opportunistic pathogens making the infections life threatening [89, 90]. Antibiotics exert selection pressure over the growth of pathogens resulting in stress driven mutation and in turn development of resistance [91, 92].

Table 7: Zones of Inhibition (mm) Showing the Bactericidal Properties of the Leaf Extract of *S. jamaicensis*

	Leaf Extract			**Synthetic Antibiotic**
				GEN
Conc. (µgml^{-1}) Organisms	**1000**	**500**	**250**	**10µg**
E. coli (-)	15	15	15	17
E. faecalis (+)	25	17	17	14
K. pneumoniae (-)	16	14	14	15
M. varians (+)	17	17	17	18
P. aeruginosa (-)	17	14	14	14
P. mirabilis (-)	17	15	15	20
S. agalactiae (+)	12	12	12	-
S. aureus (+)	14	14	14	-
S. marcescens (-)	20	20	18	30
S. typhimurium (-)	22	14	14	17

Key note: Resistant (--), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive (15–19 mm) and ultrasensitive (>20 mm)

Conclusion

S. jamaicensis leaf extract is rich in phytochemicals with proven antimicrobial and antioxidant activities. The phytochemical analysis conducted on the leaf methanolic extract revealed the presence of pharmacologically active phytochemicals. The overall therapeutic activities might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that leaves of the investigated plant could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. Consumable foods should contain natural products, which have multi-therapeutic phytochemicals that help to restore the balance between the natural antibiotics, antioxidants, free radical scavenging and enhance body defence against diseases.

Conflict of Interest Statement: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

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