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Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of *Schotia latifolia* Jacq

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## ABSTRACT

**Objective:** To investigate the antibacterial and antioxidant activities of hydroalcoholic extract of *Schotia latifolia* (*S. latifolia*) bark commonly used in South Africa traditional medicine for the treatment of various ailments. **Methods:** The antibacterial test and MIC was determined by using agar well diffusion and dilution methods respectively against eight strains of bacteria. The total phenol, proanthocyanidin and flavonoid contents of *S. latifolia* were assessed using standard methods. The antioxidant activity of the extract was evaluated using ferric reducing power and the free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic-acid (ABTS), nitric oxide (NO), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and lipid oxidation (LO). **Results:** The antibacterial activity demonstrated an appreciable effect against all the gram positive bacteria at MIC between 0.016 and 10 mg/mL while that of gram negative bacteria was above 10 mg/mL. The plant extract exhibited high concentration of proanthocyanidin [(300.00±0.10) mg CE/g], followed by flavonoid [(12.46±0.04) mg TE/g] and phenol [(11.06±0.03) mg QE/g] contents. Similarly, the extract at 0.5 mg/mL scavenges DPPH, ABTS, H<sub>2</sub>O<sub>2</sub>, LO and NO by 87.55%, 89.47%, 77.15%, 86.48% and 77.75% of the radicals respectively. The reducing power was also found to be concentration dependent. **Conclusions:** Our data suggest that *S. latifolia* extract has antibacterial and antioxidants activity and thus could be used as alternative therapy against antibiotic resistance bacteria and to prevent many radical related diseases.

## 1. Introduction

Medicinal plants are traditionally used to prevent diseases, maintain health and to cure ailments[1]. It is estimated that about 80% of the population in Africa use traditional medicine to meet their health care needs. This is because, they produce wide array of phytochemicals most of which are used by the plant as a chemical defense against predators. Plants with these compounds such as peptides, flavonoids, alkaloids, phenols and tannins have been reported to possess strong biological activities which are now gaining much importance[2]. Majority of these plants have been used in extensive application against human and animal pathogens[3]. However, drug resistance of human pathogenic bacteria has been documented globally and thus natural product from plant origin are heavily dependent by man for the treatment of diseases[4,5]. Nowadays, antioxidant

agents of plant source have attracted special interest because they can protect human body from diseases induced by free radicals with little or no side effects. This is contrary to synthetic antioxidants agents such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone which have been found to exhibit mutagenesis and liver damage in both human and animals.

*Schotia latifolia* (*S. latifolia*) Jacq (Fabaceae), known as bush boer-bean in South Africa. It is a tree which grows up to 3 m high in dry and scrubby habitat but may reach 15 m when growing in moist areas. This plant is mostly found in bush-veld, scrub, forest and forest margins of Western and Eastern Cape of South Africa[6]. Traditionally, the bark of *S. latifolia* is used for the management of heartburn, hangover and diarrhea[7]. It is also used in livestock for the management of red-water disease[8,9]. In addition, the infusion of *S. latifolia* has been reported for the treatment of obesity and other diseases such as diabetes, hypertension, chest pain and arthritis[10].

To the best of our knowledge there was no information in scientific literature on the antibacterial and antioxidant activities of hydroalcoholic stem bark extract of *S. latifolia*. Therefore, this study was designed to evaluate antibacterial

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and antioxidant activities of this plant in order to give credence to its acclaimed ethnomedicinal usage.

## 2. Materials and methods

### 2.1. Plant collection and extract preparation

The stem bark of *S. latifolia* was collected from Amathole Mountain Eastern Cape Province of South Africa in April 2010. The plant was identified and authenticated by Prof. DS Grierson of the Department of Botany, University of Fort Hare. Voucher specimen (SUNBLESS) was deposited at the Giffen herbarium of the University. The bark was oven – dried at 40 °C for 14 days, pulverized and stored in an airtight container for further use. The powdered plant material (100 g) was extracted in 1 L of 70%v/v (alcohol: water) on a mechanical shaker (Labotec Scientific Orbital Shaker, SA) for 48 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure at 40 °C to recover ethanol and later frozen at –40 °C to dryness for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA). The yield after extraction was 15.48 g which was later reconstituted in distilled water to give the required concentrations needed for this study.

### 2.2. Bacterial strains used in this study

The reference strains used in this study were chosen based on their pathological effects on human and deterioration of food products: Gram positive bacteria: *Bacillus cereus* (KZN), *Staphylococcus aureus* (OKOH1), *Bacillus pumilus* and *Mycobacteria aurum*. Gram negative bacteria: *Proteus vulgaris* (CSIR 0030), *Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 8739) and *Klebsiella pneumoniae* (KZN), were obtained from the Department of Microbiology, University of Fort Hare.

### 2.3. Antibacterial activity

The agar well diffusion based method of Deans and Ritchie<sup>[11]</sup> modified by Oyedemi *et al.*<sup>[12]</sup> was used to determine the susceptibility of bacteria. A 100 µL of 18 h bacterial cultures were used to spread a bacterial lawn on nutrient agar. The cultures were adjusted to approximately 10<sup>5</sup> CFU/mL using Mc Farland standard. Twenty five microliters (25 µL) of various concentrations of plant extracts was added to each well (diameter of 4 mm) bored on nutrient agar plate under aseptic condition. The plates were left for 30 min at room temperature for the diffusion of the extracts and incubated at 37 °C for 18 h. The zones of inhibition were measured after 18 h using ruler. Each concentration of the extract was repeated three times. The minimum inhibitory concentration of *S. latifolia* extract against the test bacteria was determined by agar dilution method as described by the National Committee for Clinical Laboratory Standards<sup>[13]</sup>. The MICs were the lowest concentrations of the extract resulting to complete inhibition of visible growth of the test organisms.

### 2.4. Total phenols contents

The amount of phenolics compound in the hydroalcoholic stem bark extract of *S. latifolia* was determined with

Folin Ciocalteu reagent using the method of Spanos and Wrolstad<sup>[14]</sup> modified by Zovko<sup>[15]</sup>. To 0.5 mL of plant extract solution (1 mg/mL) was added 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of Na<sub>2</sub>CO<sub>3</sub> (2% w/v). The resulting mixture was incubated at 45 °C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible light. Total phenolics content was expressed as mg/g tannic acid equivalent using the following equation from the calibration curve:  $Y = 0.1216x$ ,  $R^2 = 0.9365$ , where  $x$  was the absorbance and  $Y$  was the tannic acid equivalent (mg/g). The experiment was conducted in triplicate, and the results are reported as mean±SD values.

### 2.5. Total flavonoids contents

Total flavonoids content in the extracts was determined by aluminum colorimetric assay<sup>[16]</sup> with some modifications. One milliliter (1 mL) of sample (1 mg/mL) was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. All determinations were done in triplicate and values were calculated from calibration curve obtained from quercetin using the following equations:  $Y = 0.0255x$ ,  $R^2 = 0.9812$ , where  $x$  was the absorbance and  $Y$  the quercetin equivalent (mg/g).

### 2.6. Total proanthocyanidins contents

Total proanthocyanidins was determined based on the procedure of Sun *et al.*<sup>[17]</sup>. The mixture of 3 mL of vanillin–methanol (4% v/v), 1.5 mL of hydrochloric acid was added to 0.5 mL (1 mg/mL) of aqueous extract and vortexed. The resulting mixture was allowed to stand for 15 min at room temperature and the absorbance was then measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the equation obtained from the calibration curve:  $Y = 0.5825x$ ,  $R^2 = 0.9277$ , where  $x$  was the absorbance and  $Y$  is the catechin equivalent (mg/g).

### 2.7. Determination of reducing power

The reducing power of the extract was evaluated according to the method of Yen and Chen<sup>[18]</sup> with some modifications. To 1.0 mL of the extract, butylated hydroxytoluene (BHT), ascorbic acid and alpha tocopherol (vitamin E) was mixed to the mixture containing 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% K<sub>3</sub>Fe(CN)<sub>6</sub>. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of 10% TCA. Exactly 2.5 mL of the solution after vigorous shaking was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>. The absorbance was measured at 700 nm against blank sample.

### 2.8. DPPH radical scavenging activity

The method described by Liyana–Pathiranan and Shahidi<sup>[19]</sup> was used to determine DPPH scavenging activity of the plant extract. A solution of 0.135 mM DPPH was prepared in methanol. One milliliter (1 mL) of the solution was mixed with 1.0 mL of the extract, BHT and rutin (0.025–0.5 mg/mL) prepared in methanol which are used as reference drugs. The reaction mixture was vortexed thoroughly and left in the dark for 30 min at room

temperature. The absorbance of the mixture was measured at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of DPPH radical+methanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of DPPH radical+sample extract/standard.

### 2.9. ABTS radical scavenging activity

Ability of plant extract to scavenge ABTS radical was assessed using the method of Re *et al*[20]. The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate solution in equal amount and allowed to react for 12 h at room temperature in the dark. The resulting solution was later diluted by mixing 1 mL of freshly prepared ABTS radical solution to obtain an absorbance of  $0.706 \pm 0.001$  units at 734 nm after 7 min. The percentage inhibition of ABTS radical by the extract, BHT and rutin was calculated and compared with that of BHT and rutin following the equation:

$$\text{ABTS radical scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of ABTS radical+methanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of ABTS radical+sample extract/standard.

### 2.10. Nitric oxide scavenging activity

The method of Ebrahimzadeh *et al*[21] was adopted to determine the scavenging activity of *S. latifolia* extract against nitric oxide radical. A total of 2 mL sodium nitroprusside (10 mM) was prepared in 0.5 mM phosphate buffer saline (pH 7.4) mixed with 0.5 mL of plant extract, BHT and rutin at various concentrations (0.025–0.5 mg/mL). The amount of nitric oxide radical inhibited by the extract was calculated using this equation:

$$\text{Nitric oxide radical scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of nitric oxide radical + methanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of nitric oxide radical+sample extract/standard.

### 2.11. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the plant extract was estimated using the modified method described by Oyedemi *et al*[2]. Plant extract (stock 4 mg/mL) prepared in distilled water at various concentrations was mixed with 0.6 mL of 4 mM  $\text{H}_2\text{O}_2$  dissolved in phosphate buffer (0.1 M pH 7.4) was incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without  $\text{H}_2\text{O}_2$ . The amount of  $\text{H}_2\text{O}_2$  inhibited by the extract was calculated using the following equation:

$$\text{H}_2\text{O}_2 \text{ radical activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of  $\text{H}_2\text{O}_2$  +methanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of  $\text{H}_2\text{O}_2$  + sample or extract or standard.

### 2.12. Estimation of lipid peroxidation

A modified thiobarbituric acid–reactive species (TBARS) assay[22] was used to measure the lipid peroxide formed using egg–yolk homogenates as lipid–rich media[23]. Ten percent of egg homogenate prepared in distilled water (0.5 mL)

with 0.1 mL of stem bark extract of *S. latifolia* (0.025–0.5 mg/mL) were mixed in a test tube and then made up to 1 mL with distilled water. Precisely, 0.05 mL of 0.07M  $\text{FeSO}_4$  was added to the above mixture and later incubated for 30 min, to induce lipid oxidation. Thereafter, 1.5 mL of 20% acetic acid (pH 3.5), 1.5 mL of 0.8% TBA (w/v) prepared in 1.1% sodium dodecyl sulphate was mixed together with 0.05 mL of 20% TCA vortexed. The resulting mixture was heated in a boiling water bath for 60 min. After cooling, 5.0 mL of 1–butanol was added and later centrifuged at 3 000 rpm for 10 min. The absorbance of the upper layer solution was measured at 532 nm. For the blank 0.1 mL of distilled water was used instead of the extract.

### 2.13. Statistical analysis

Data were expressed as means $\pm$ SD (standard deviation) of five replicates and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS. Values were considered significant at  $P < 0.05$ .

## 3. Results

### 3.1. Antibacterial testing

The hydroalcoholic extract of stem bark of *S. latifolia* was tested for antibacterial activity against eight strains of bacterial pathogens by using serial dilution of 10 to 0.3125 mg/mL. The plant extract showed antibacterial activity against gram positive bacteria: *Bacillus cereus* (KZN), *Staphylococcus aureus* (OKOH1), *Bacillus pumilus* and *Mycobacteria aurum* with the zone of inhibition of 17, 15, 15 and 14 mm, respectively (Table 1). At 10 mg/mL, the extract exhibited antibacterial activity against *Escherichia coli* (ATCC 8739) and *Klebsiella pneumoniae* (ATCC 4352) with zone inhibition of 10 and 12 mm, respectively but not effective against *Proteus vulgaris* (CSIR 0030) and *Pseudomonas aeruginosa* (ATCC 19582). The plant extract exhibited antibacterial activity against *Bacillus cereus* (KZN), *Staphylococcus aureus* (OKOH1), *Bacillus pumilus* and *Mycobacteria aurum* at the MIC of 0.625, 10, 2.5 and 10 mg/mL, respectively while that of gram negative bacteria was  $\geq 10$  mg/mL.

### 3.2. Total polyphenolics assay

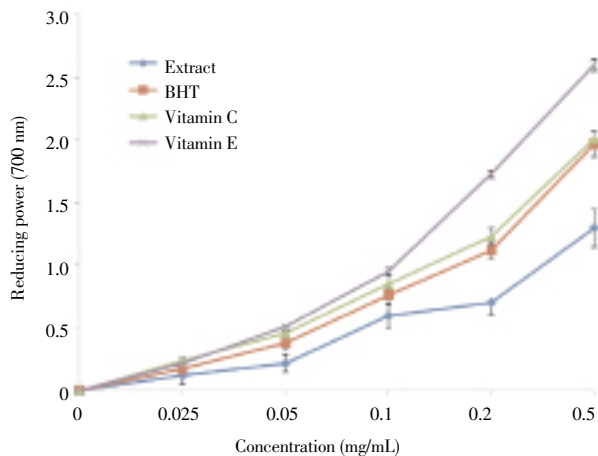
The present study revealed high concentration of proanthocyanidins [ $300.00 \pm 0.10$  mg CE/g], followed by flavonoids [ $12.46 \pm 0.04$  mg TE/g] and phenols [ $11.06 \pm 0.03$  mg QE/g] contents. The presence and synergistic potential of these compounds could be responsible for the potent antioxidant and antibacterial activities of hydroalcoholic extract of *S. latifolia* observed in this study. Total phenolics content was expressed as tannic acid equivalent while both proanthocyanidins and flavonoids were expressed as quercetin equivalent.

### 3.3. Antioxidant assays

#### 3.3.1. Reducing power assay

Figure 1 shows concentration–response curves for the reducing power of the plant extract, vitamin E, vitamin C

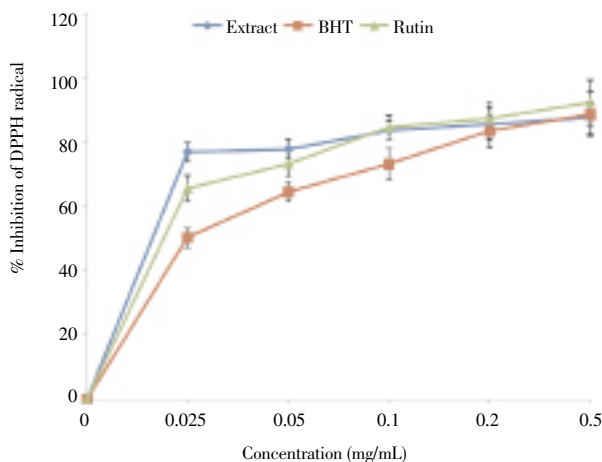
and BHT. The antioxidant activity of the extracts which were reflected through reductive ability of Fe<sup>3+</sup> to Fe<sup>2+</sup> was increased with increasing concentration. The antioxidant activity of the extracts was significantly lower than the standard drugs in this order: vitamin E > vitamin C > BHT > extract.



**Figure 1.** Ferric reducing activity of hydroalcoholic stem bark extract of *S. latifolia* and the standard BHT, vitamin C and vitamin E.

### 3.3.2. DPPH scavenging activity

The DPPH radical scavenging ability of plant extract, BHT and rutin is shown in Figure 2. The plant extract showed a significant DPPH radical scavenging activity comparable to BHT and rutin standard antioxidant drugs used in this study. At 0.5 mg/mL, the percentage scavenging of DPPH radical was 87.6%, 88.5% and 92.2% for the extract, BHT and rutin while the IC<sub>50</sub> was found to be 0.016, 0.023 and 0.019 mg/mL, respectively.

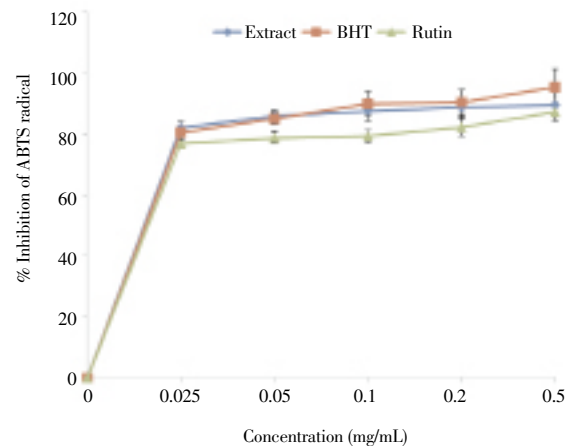


**Figure 2.** The DPPH radical scavenging activity of *S. latifolia* extract and the standard rutin and BHT.

### 3.3.3. ABTS scavenging activity

The result obtained from this assay revealed the high level of proton radical scavenging ability of the plant extract as presented in Figure 3. Plant extracts at 0.5 mg/mL scavenged ABTS radical by 89.47% which was significantly different from that of BHT (95.50%) but higher than rutin (87.42%). IC<sub>50</sub> values for extract, BHT and rutin were found to be 0.015,

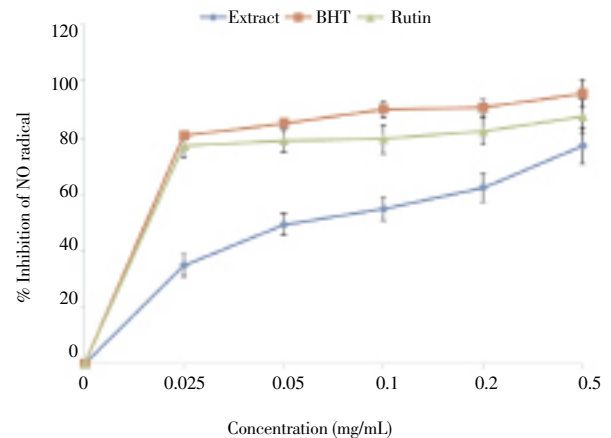
0.015 and 0.016 mg/mL, respectively.



**Figure 3.** The ABTS radical scavenging activity of *S. latifolia* extract and the standard rutin and BHT.

### 3.3.4. Nitric oxide scavenging activity

Figure 4 shows the nitric oxide scavenging activity of plant extract and the reference drugs used in this study. The scavenging activity of the extract against nitric oxide was near that of rutin but significantly lower as compared with BHT. Percentage scavenging activity of extract, rutin and BHT against nitric oxide was 77.15%, 82.30% and 94.50%, respectively. The IC<sub>50</sub> value for the extract, rutin and BHT was 0.072, 0.015 and 0.016 mg/mL, respectively. Though the IC<sub>50</sub> value of the extract was significantly lower than reference drugs but it possessed strong scavenging activity against nitric oxide radical which was reflected in percentage radical inhibited.

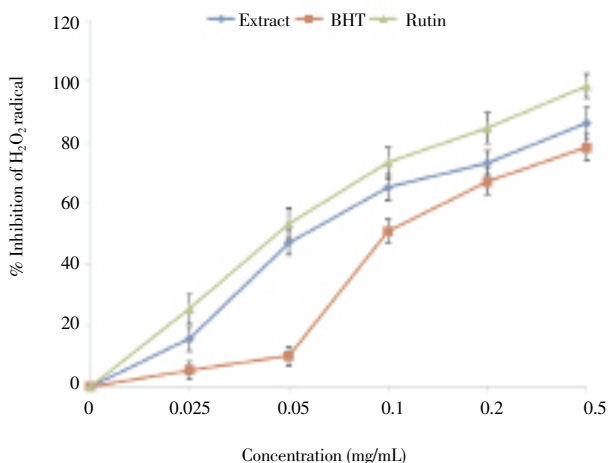


**Figure 4.** The nitric oxide radical scavenging activity of *S. latifolia* extract, rutin and BHT.

### 3.3.5. Hydrogen peroxide scavenging activity

The ability of hydroalcoholic extract of *S. latifolia* to scavenge hydrogen peroxide radical is presented in Figure 5 using BHT and gallic acid as positive controls. The results indicated a concentration dependent activity with percentage inhibition of 86.48%, 78.53% and 96.82% for plant extract, BHT and gallic acid, respectively at 0.5 mg/mL. The plant extract had more power to eliminate hydrogen

peroxide which was depicted with IC<sub>50</sub> value of 0.066 mg/mL with greater activity than BHT (0.096 mg/mL) but lower as compared with gallic acid (0.047 mg/mL).

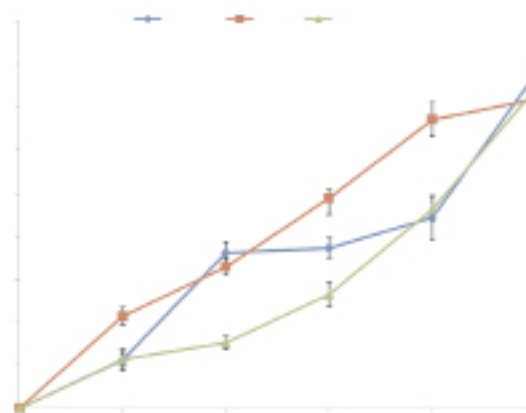


**Figure 5.** The hydrogen peroxide radical scavenging activity of *S. latifolia* extract and the standard rutin and BHT.

**3.3.6. Lipid peroxidation scavenging activity**

The results in Figure 6 showed the scavenging capacity of lipid peroxidation by the extract, BHT and gallic acid

was found to have IC<sub>50</sub> values of 0.24, 0.12 and 0.24 mg/mL, respectively. At 0.5 mg/mL, the percentage inhibition was 77.75%, 72.00% and 74.39% for the extract, BHT and gallic acid. The plant extract showed a good capacity of depleting lipid peroxidation that is comparable to the standard gallic acid and higher than BHT.



**Figure 6.** Percentage inhibition of lipid peroxidation of *S. latifolia* extract and the standard rutin and BHT.

**Table 1**

Antibacterial activity of hydroalcoholic stem bark extract of *S. latifolia*.

Bacteria	Gram (+/-)	Zone of inhibition (mm)					CHLRP (μg/mL)	STREPT (μg/mL)
		0.625 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL		
<i>Bacillus cereus</i> (KZN)	+	17	19	20	22	24	<2	<2
<i>Staphylococcus aureus</i> (OKOH1)	+	na	na	na	8	15	<2	<2
<i>Bacillus pumilus</i>	+	na	12	15	17	22	<2	<2
<i>Mycobacteria aurum</i>	+	na	na	7	10	14	<4	<4
<i>Proteus vulgaris</i> (CSIR 0030)	-	na	na	na	na	7	<2	<4
<i>Pseudomonas aeruginosa</i> (ATCC 19582)	-	na	na	na	na	6	<10	<4
<i>Escherichia coli</i> (ATCC 8739)	-	na	na	na	na	10	<2	<2
<i>Klebsiella pneumoniae</i> (ATCC 4352)	-	na	na	na	8	12	<2	<2

CHLRP stands for chloramphenicol; STREPT for streptomycin and na stands for data not available.

**4. Discussion**

The present study indicated potent antibacterial activity of *S. latifolia* extract towards gram positive bacteria than the gram negative bacteria in agreement to various scientific reports[24,25]. The difference observed have been reported in several studies of medicinal plants due to cell wall composition of gram positive bacteria which is of a single layer structure whereas gram negative bacteria is multilayered structure[26]. The inhibitory effect of the extract was very significant particularly against gram positive bacteria which could be attributed to the presence of phenols, flavonoids and proanthocyanidins. These compounds have been reported to possess strong biological activities against some pathogenic bacterial[27].

Proanthocyanidins is a polymer of flavonoids and has been reported to possess strong antioxidant properties with

numerous biological activities[28]. The compound is used to lower blood pressure, emolliate blood vessels, prevent blood vessel scleroses and thrombus formation[29]. The antioxidant and free radical scavenging activity of this compound has also been demonstrated in many experimental studies to twice vitamins E and four times as potent as vitamin C[30,31]. Inhibitory effect of proanthocyanidins against lipid oxidation and platelet aggregation in man has also been documented[32]. Therefore high concentration of proanthocyanidins together with synergistic effect of phenols and flavonoids could be accountable for the potent antioxidant and free radicals scavenging activity observed in this present study.

The reducing power of *S. latifolia* extract was used to determine the possible antioxidant activity by measuring the transformation of Fe<sup>3+</sup> to Fe<sup>2+</sup> [33]. The reductive capability of this plant was monitored by the formation of blue colour

at 700 nm. The changes of yellow color to blue or green of the test solution could be attributed to the concentration and reductive ability of the extract. The reducing value of the extracts was significantly lower than the standard drugs in this order: vitamin E > vitamin C > BHT > extract. The data obtained from this study suggested that the extracts are electron donors and thus could possibly reduce the oxidized intermediates of lipid peroxidation in comparison to standard antioxidant drugs<sup>[34]</sup>. The presence of reductones in plant extracts have been reported in several studies to contribute significantly for the termination of free radical chain reactors as shown in this study<sup>[35]</sup>.

DPPH assay is a simple method commonly used to survey antioxidant activity of a specific compound or plant extracts. The scavenging activity of extract against DPPH radical showed a comparative effect with BHT but lower than the standard rutin. Our results demonstrated potent antioxidant activity against DPPH<sup>•</sup> which could be related to high proanthocyanidin content. Similarly, the moderate phenols and flavonoids contents could enhance hydrogen proton donation to the unpaired electrons of the radical, chelating of transition metals thus neutralize its pathological damage<sup>[36]</sup>. The data obtained from this study concurred with the findings of Oyedemi *et al*<sup>[16]</sup> but contradict with the report of Afolayan *et al*<sup>[37]</sup>. It could be inferred from this study that *S. latifolia* extract could serve as a potential source of natural antioxidant agent against radical related diseases.

ABTS assay was carried out to gain broad knowledge of antioxidant activity of the plant extract due to different solubility or testing systems of the reacting solution. Total antioxidant potential of the extract was measured based on the decolorization of blue chromophore of ABTS radical formed by the reaction of ABTS and potassium persulphate. The reaction of plant extract or standard drugs (BHT and rutin) to the pre-formed radical cation converts it to ABTS in a concentrations related manner. Our result from this assay confirmed the antiradical activity of extract against DPPH radical which is comparable with the standard antioxidant drug. Eradication of this radical and DPPH<sup>•</sup> confirmed the oxidizing potential of plant extract against proton radical generated within the system<sup>[38]</sup>.

Nitric oxide is an important chemical mediator generated by endothelial cell and macrophages but play a crucial role in the pathogenesis of various diseases caused by inflammation when combined with superoxide radical to form peroxynitrite anion<sup>[39]</sup>. This radical is generated from sodium nitroprusside in aqueous solution and reacts with oxygen to form nitrite. The scavenging activity of the extract against nitric oxide was near that of rutin but significantly ( $P < 0.05$ ) lower as compared with BHT. Our data therefore corroborated with the report of several researchers<sup>[37,40]</sup> but contradicted with our previous findings on *Strychnos henningsii* which showed a moderate activity. The percentage inhibition displayed by the extract showed that the extract was a potent scavenger of nitric oxide as a result could be a potential agent to prevent initiation of chain reactions that are detrimental to human health.

The conversion of hydrogen peroxide to hydroxyl radical may be toxic to the cell. It is an extremely reactive free radical formed in biological systems and known to implicate

highly damaging species in free radical pathology. Among the oxygen radicals, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules<sup>[41]</sup>. Therefore, eradication of hydrogen peroxide is very important in order to defend the cell or body system from invading agents. Scavenging activity of *S. latifolia* against hydrogen peroxide was concentration dependent which is comparable to standard antioxidant drugs used in this study. This finding is correlated with high proanthocyanidins content together with flavonols and phenolic contents.

Free radicals induced peroxidation in polyunsaturated lipid which occurs either through ferryl-perferryl complex or through OH<sup>•</sup> radicals<sup>[42]</sup>. In this study, lipid peroxidation was brought about by egg-yolk homogenates as lipid-rich media. The plant extracts at 0.5 mg/mL showed strong antioxidant activity by lowering lipid peroxides formation which is indicated by their high absorbance values. The activity is concentration dependent against lipid peroxide generation and could be linked to high content of polyphenolic compounds that have been implicated with strong antioxidant activity.

Generally, there is enough evidence to support the biological activities of hydroalcoholic extract of *S. latifolia* bark observed in this present study. The presence of phenolic compounds and high content of proanthocyanidins have been reported in several studies to be responsible for this observation. The data obtained from this study has provided scientific credence to the traditional usage of *S. latifolia* in South Africa traditional medicine. However further studies are needed to understand the underlying mechanisms of action of this plant for proper elucidation of its biological activities.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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