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DPPH RADICAL SCAVENGING ACTIVITY OF EXTRACTS FROM *BUDDLEJA SALVIIFOLIA*

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Abstract

Hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *Buddleja salviifolia* were screened for their antioxidant activity by DPPH radical scavenging assay. *B. salviifolia* hexane leaves extract, *B. salviifolia* chloroform leaves extract, *B. salviifolia* ethyl acetate leaves extract and *B. salviifolia* methanolic leaves extract showed scavenging activity in the range of 10.04±3.97 to 17.45±3.64, 41.73±1.68 to 47.46±1.36, 25.81±1.34 to 47.43±4.25 and 44.88±1.50 to 50.34±3.09%, respectively, at various concentrations. *B. salviifolia* hexane stem-bark extract, *B. salviifolia* chloroform stem-bark extract, *B. salviifolia* ethyl acetate stem-bark extract and *B. salviifolia* methanolic stem-bark extract showed scavenging activity in the range of 2.51±1.22 to 32.30±3.16, 27.90±3.74 to 51.91±1.43, 5.30±2.13 to 34.89±3.59 and 19.86±3.23 to 57.24±1.23%, respectively, at various concentrations. The IC_{50} values of all these extracts were also determined and found to be in the range of 450 to >3000 µg/mL. The positive control, ascorbic acid, showed an IC_{50} value of <200 µg/mL.

Keywords: *Buddleja salviifolia, Scrophulariaceae, radical scavenging activity, DPPH assay, ascorbic acid, hexane extracts, methanolic extracts.*

Introduction

Known by other names such as sagewood, wildsaile and saliehout, *Buddleja salviifolia* belongs to the Scrophulariaceae family of the *Buddleja* genus [1-3]. *B. salviifolia* is a bushy shrub with a greyish appearance [2,4]. The existence of small trees of 3-8 meter height with many-stems at base have also been identified [2,4]. Approximately, 150 species areknown in the genus *Buddleja* and are widely distributed in various parts of the Southern Africa such as South Africa, Lesotho, Swaziland and Zimbabwe. [5]. *B. salviifolia* commonly found in rocky mountain slopes, dry hillsides, forest margins, coastal and along the watercourses [6]. *B. salviifolia* has small, tubular flowers with enclosing stamens. It has a strong and sweet perfumed smell, especially in the early spring [6]. Basotho tribes have traditionally been used the leaves of *B. salviifolia* as herbal remedy to improve digestion, as an anti-emetic and to alleviate constipation [7]. The leaves of *B. salviifolia* have also been used to relief colic, nausea and coughs [8] and to treat eye infections and neurodegenerative conditions in South Africa [9]. In the traditional medicine, *B. salviifolia* has been used to treat TB, syphilis, herpes, cervical cancer and complications associated with pregnancy. Pure compounds such as 4-hydroxyphenyl ethyl vanillate, acteoside and quercetin have been isolated from ethyl acetate leaves extract of *B. salviifolia* [9,10] and these pure compounds have also been evaluated for their antioxidant activity [9,10]. To the best of our knowledge, *B. salviifolia* has not been explored well for their biological and pharmacological activities, particularly from the species from the Kingdom of Lesotho. The aim of the present study was to evaluate the antioxidant activity of hexane, chloroform, ethyl acetate and methanolic extracts from the leaves and stem-bark of *B. salviifolia* collected from the Kingdom of Lesotho using DPPH free radical scavenging assay. The results are communicated in this article.

Methods

Chemicals and reagents

Hexane (AR grade, 99.5%), chloroform (AR grade, 99.5%), ethyl acetate (AR grade, 99%) and methanol (AR grade, 99.5%) were all purchased from Sigma-Aldrich. Ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Prestige Laboratory Supplies.

Plant materials

Fresh leaves of *B. salviifolia* collected in August 2018 inside the Roma Campus of National University of Lesotho, Lesotho, Southern Africa. Additionally, a piece of stem-bark cut from the same plant. A voucher specimen viz. Matamane/BSLS/2018 and Matamane/BSSB/2018 for leaves and stem-bark, respectively, were kept separately in the Organic Research Laboratory, Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, Maseru, the Kingdom of Lesotho, Southern Africa.

Processing of plant materials

The leaves were allowed to air-dry at room temperature for two weeks. The crushed leaves were ground into powder (523.86g) using a laboratory blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 3.5 AMPs, Laboratory and Analytical Supplies). The chopped stem-bark was allowed to air-dry at room temperature for two weeks and then ground into powder (760.46g) using the same blender.

Preparation of plant extracts

100.1147g of powdered leaves was extracted with hexane for three days at room temperature. The solution was filtered off using a vacuum filter (ATB, Model: 284065–H, Power: 230V 3.0A, 1320/min 50Hz). The solvent was removed in *vacuo*. The extract was transferred to pre-weighed clean and dry beaker. The procedure was repeated twice. Finally, the leaves were extracted with hot hexane for 10 hours. 1.8590 g of combined hexane extract was obtained after removal of solvent. The same procedure was repeated with chloroform, ethyl acetate and methanol separately. 7.4851, 8.1365 and 14.6128 g of chloroform, ethyl acetate and methanolic leaves extracts, respectively, were obtained from 98.8810, 100.2324 and 175.4500g of powdered leaves. Similarly, using the same procedure, 0.4379, 1.0785, 2.1884 and 8.8601g of hexane, chloroform, ethyl acetate and methanolic stem-bark extracts, respectively, were obtained

from 200.0964, 200.0141, 202.5295 and 157.8224g of powdered stem-bark.

DPPH radical scavenging assay and determination of IC_{50} values

Antioxidant activity

The antioxidant activity of the extracts was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described in literature [11]. Briefly, a stock solution of the methanolic extract was prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Serial dilutions were made from this stock solution to obtain solutions with concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200 µg mL^{-1}. Solutions without extract concentration served as negative control. A solution of 3.94 mg of DPPH in 100 mL of methanol served as oxidant. It was prepared just before use and stored in dark to minimize degradation. 0.1 mL sample of plant extract solution was mixed with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCL buffer (pH 7.4). Similarly, stock solutions of hexane, chloroform and ethyl acetate extracts were prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Further dilutions were made from these stock solutions to obtain solutions with concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200 µg mL^{-1}. 0.1 mL each of extract was mixed separately with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCL buffer (pH7.40). A stock solution of ascorbic acid (0.3g) in 50% methanol (v/v) was prepared and serial dilutions were made as previously and served as positive control [10, 12]. 0.1 mL of ascorbic acid was mixed with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCL buffer (pH7.40). The mixtures were incubated for 30 minutes and their optical density was measured at 517 nm. Percentage inhibition of DPPH free radical was calculated using equation given below.

$$DPPH\ Scavenged\ (\%) = [(A_{cont} - A_{test})/A_{cont}] \times 100$$

A_{cont} = *Absorbance of negative control.*
A_{test} = *Absorbance in the presence of extract or positive control [14].*

The IC_{50} value is defined as the concentration (in µg mL^{-1}) of extract that inhibits the formation of DPPH radical by 50% [13]. A lower value of IC_{50} represents higher antioxidant activity. The IC_{50} values were calculated from graphs by plotting extract concentrations vs percentage inhibition of DPPH radical using Microsoft Excel. Each experiment was carried out in triplicate and the averages of the three values were used to calculate IC_{50} values. Standard deviation was calculated for each concentration from the three values of the experiment.

Statistical analysis

Results were expressed as means of three determinations. One-way analysis of variance (ANOVA) was used to compare means at the significance level $p < 0.05$. All analysis was performed by Microsoft Excel software.

Results

Table 1 summarizes the DPPH radical scavenging potential of *B. salviifolia* hexane leaves extract (BSHXLS), *B. salviifolia* chloroform leaves extract (BSCHLS), *B. salviifolia* ethyl acetate leaves extract (BSEALS), *B. salviifolia* methanolic leaves extract (BSMELS), *B. salviifolia* hexane stem-bark extract (BSHXSB), *B. salviifolia* chloroform stem-bark extract (BSCHSB), *B. salviifolia* ethyl acetate stem-bark extract (BSEASB) and *B. salviifolia* methanolic stem-bark extract (BSMESB). Ascorbic acid in 50% methanol served as positive control for all extracts. BSHXLS showed scavenging activity of 10.04±3.97, 10.68±1.24, 11.51±2.19, 13.65±4.42, 14.92±2.59, 16.14±4.74 and 17.45±3.64% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL respectively. The positive control showed scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. BSCHLS showed scavenging activity of 41.73±1.68, 42.96±1.01, 43.43±1.97, 45.94±2.91, 46.34±0.38, 47.18±1.24 and 47.46±1.36% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL respectively. The positive control showed the scavenging activities of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44%, respectively, at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. BSEALS exhibited scavenging activity

of 25.81±1.34, 30.04±2.11, 35.89±4.57, 38.56±1.79, 40.51±4.09, 44.06±2.45 and 47.46±1.36% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control showed scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. BSMELS showed scavenging activity of 44.88±1.50, 45.54±2.91, 47.17±2.33, 48.02±1.25, 48.18±2.33, 48.19±1.71 and 50.34±3.09% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control showed scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. In general, all four leaves extracts from *B. salviifolia* showed lower radical scavenging activity than the positive control at all concentrations. However, BSCHLS and BSMELS showed significantly stronger radical scavenging activity at all concentrations relative to other two extracts, BSHELS and BSEALS.

BSHXSB showed scavenging activity of 2.51±1.22, 4.95±2.30, 9.36±4.19, 10.76±3.82, 12.80±3.40, 19.36±4.15 and 32.30±3.16% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control showed scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. BSHXSB showed a lower scavenging activity than the positive control at all concentrations. BSCHSB showed scavenging activity of 27.90±3.74, 42.64±3.51, 43.83±1.42, 46.71±2.46, 49.64±3.67, 51.42±5.33 and 51.91±1.43% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL respectively. The positive control exhibited the scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result showed that at lower concentrations, BSCHSB exhibited relatively weak antioxidant activity while at higher concentrations such as 2000 and 3000 µg/mL it showed comparable scavenging activity as that of positive control. BSEASB showed scavenging activity of 5.30±2.13, 17.14±4.28, 22.61±3.09, 22.70±3.46, 32.73±6.22, 34.51±4.76 and34.89±3.59% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control exhibited scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. BSEASB showed relatively a very weak antioxidant activity. BSMESB showed scavenging activity of 19.86±3.83, 54.94±0.64, 55.23±1.23, 56.24±1.23, 56.72±0.36, 56.74±2.03 and 57.24±1.23% respectively at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control exhibited scavenging activity of 53.03±3.98, 53.46±0.14, 53.82±1.22, 53.84±4.30, 54.11±0.99, 54.34±0.92 and 56.45±5.44% at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL respectively. At a concentration of 200 µg/mL, BSMESB showed a weak scavenging activity with 19.86±3.83% inhibition. However, at higher concentrations such as 500, 800, 1000, 1500, 2000 and 3000 µg/mL, BSMESB showed a strong scavenging activity (refer to Table **1**).

Table 2 summarizes the IC_{50} values of various leaves and stem-bark extracts of *B. salviifolia*. The IC_{50} values of BSHXLS, BSCHLS, BSEALS, BSMELS, BSHXSB, BSCHSB, BSEASB and BSMESB were found to be >3000, >3000, ~3000, 2250, >3000, 1592.21, >3000 and 450 µg/mL, respectively (refer to Table **2**). BSMESB was the most potent among all extracts with IC_{50} value of 450 µg/mL. The positive control showed an IC_{50} value of <200 µg/mL. For comparison and clarity, the percentage of radical scavenging activity of various extracts from leaves and stem-bark of *B. salviifolia* at various concentrations are shown in Figures **3** and **4**. The percentage of radical scavenging activity is increased with increasing extract concentrations. In other words, the antioxidant activity of extracts is dose-dependent.

Discussion

4-hydroxyphenyl ethyl vanillate, acteoside and quercetin have been isolated from ethyl acetate extract of leaves of *B. salviifolia* and they showed IC_{50} values of 17.94±0.0, 4.28±1.13 and 5.08±0.0, respectively, in the DPPH radical scavenging assay [9,10]. They also showed EC_{50} values of 03±13.13,

63.30±17.66 and 202.06±34.37 µg/mL, respectively [9]. Dichloromethane-methanol (1:1) and water extracts obtained from whole plant of *B. salviifolia* showed IC_{50} values of 0.23±0.01 and 1.60±0.51 mg/mL, respectively, in the DPPH radical scavenging assay [15] and 0.14±0.08 and 1±0.05 mg/mL, respectively, in the ABTS assay [15]. Additionally, the dichloromethane-methanol (1:1) extract has also been evaluated for its acetylcholinesterase inhibitory activity and showed an IC_{50} value of 0.05±0.02 mg/mL. Acteoside reported to have a strong anti-leukemic and cytotoxic activity against murine cell lines and anti-inflammatory activity [16, 17]. Dichloromethane bark extract from *B. salviifolia* has a promising anti-inflammatory activity and moderate antiplasmodial activity [18].

We evaluated the DPPH radical scavenging activity of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *B. salviifolia* collected from the Kingdom of Lesotho. The DPPH radical scavenging activity of the hexane, chloroform, ethyl acetate and methanolic leaves extracts were found to be in the ranges of 10.04±3.97 to 17.45±3.64, 41.73± to 47.46±1.36, 25.81±1.36 to 47.43±4.25 and 44.88±1.50 to 50.34±3.09% respectively. The radical scavenging activity of hexane, chloroform, ethyl acetate and methanolic stem-bark extracts were found to be in the ranges of 2.51±1.22 to 32.30±3.16, 27.90±3.74 to 51.91±1.43, 5.30±2.13 to 34.89±3.59 and 19.86±3.23 to 57.24±1.23%, respectively. The IC_{50} values of all these extracts were also determined and found to be in the range of 450 to >3000 µg/mL. BSMESB was the most potent among all extracts with IC_{50} value of 450 µg/mL. From this study, we concluded that extracts from leaves and stem-bark of *B. salviifolia* exhibited a significant free radical scavenging activity. *B. salviifolia* finds its therapeutic applications in the traditional medicine to treat variety of diseases. Therefore, further studies on this plant will be useful to commercialize products from this plant.

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Table 1. The percentage inhibition of DPPH radical by various leaves and stem-bark extracts of *B. salviifolia* at various concentrations.

Extracts	Concentrations (µg/mL)/ (%) Inhibition						
	200	500	800	1000	1500	2000	3000
BSHXLS	10.04±3.97	10.68±1.24	11.51±2.19	13.65±4.42	14.92±2.59	16.14±4.74	17.45±3.64
BSCHLS	41.73±1.68	42.96±1.01	43.43±1.97	45.94±2.91	46.34±0.38	47.18±1.24	47.46±1.36
BSEALS	25.81±1.34	30.04±2.11	35.89±4.57	38.56±1.79	40.51±4.09	44.06±2.45	47.43±4.25
BSMELS	44.88±1.50	45.54±2.91	47.17±2.33	48.02±1.25	48.18±2.33	48.19±1.71	50.34±3.09
BSHXSB	2.51±1.22	4.95±2.30	9.36±4.19	10.76±3.82	12.80±3.40	19.36±4.15	32.30±3.16
BSCHSB	27.90±3.74	42.64±3.51	43.83±1.42	46.71±2.46	49.64±3.67	51.42±5.33	51.91±1.43
BSEASB	5.30±2.13	17.14±4.28	22.61±3.09	22.70±3.46	32.73±6.22	34.51±4.76	34.89±3.59
BSMESB	19.86±3.83	54.94±0.64	55.23±1.23	56.24±1.23	56.72±0.36	56.74±2.03	57.24±1.23
Asc. acid	53.03±3.98	53.46±0.14	53.82±1.22	53.84±4.30	54.11±0.99	54.34±0.92	56.45±5.44

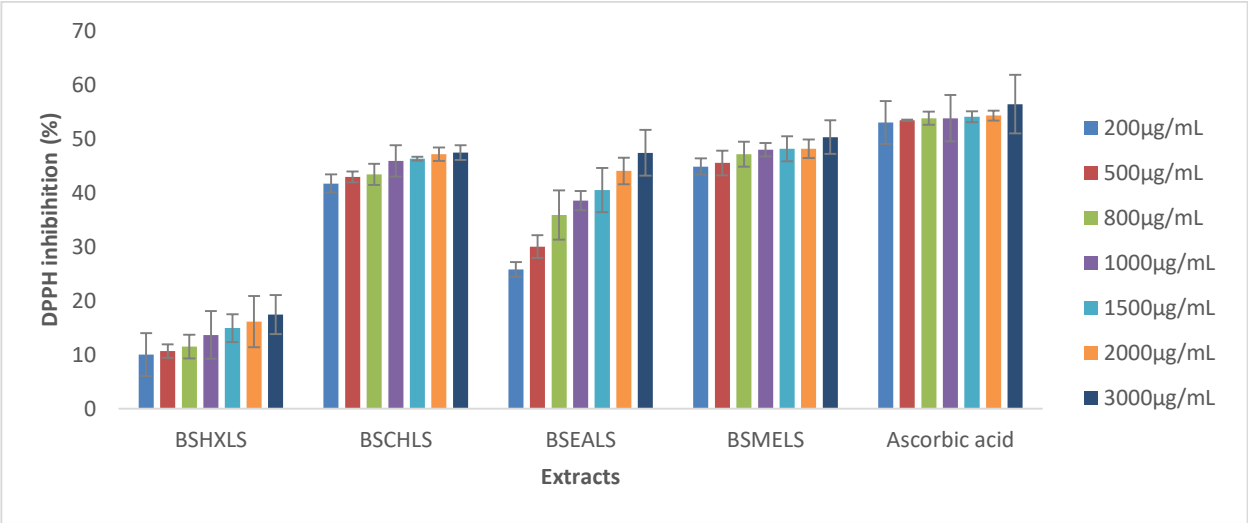
BSHXLS = *B. salviifolia* hexane leaves extract, BSCHLS = *B. salviifolia* chloroform leaves extract, BSEALS = *B. salviifolia* ethyl acetate leaves extract, BSMELS = *B. salviifolia* methanol leaves extract, BSHXSB = *B. salviifolia* hexane stem-bark extract, BSCHSB = *B. salviifolia* chloroform stem-bark extract, BSEASB = *B. salviifolia* ethyl acetate stem-bark extract, BSMESB = *B. salviifolia* methanol stem-bark extract; Asc**.** acid = Ascorbic acid which served as positive control. The experiments were carried out in triplicates and each value is expressed as mean±standard deviation (*n*=3).

Table 2. The IC_{50} values of various leaves and stem extracts from *B. salviifolia*

S/N	Extracts	IC_{50} (µg/mL)
1	BSHXLS	>3000
2	BSCHLS	>3000
3	BSEALS	~3000
4	BSMELS	2250
5	BSHXSB	>3000
6	BSCHSB	1592.21
7	BSEASB	>3000
8	BSMESB	450
9	Asc. acid	<200

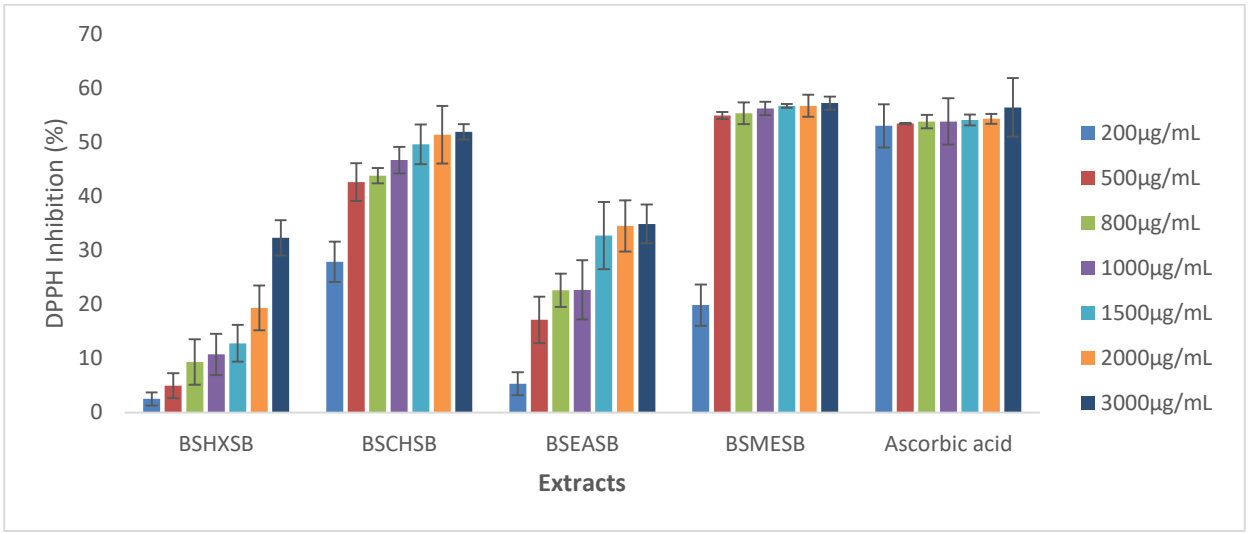
BSHXLS = *B. salviifolia* hexane leaves extract, BSCHLS = *B. salviifolia* chloroform leaves extract, BSEALS = *B. salviifolia* ethyl acetate leaves extract, BSMELS = *B. salviifolia* methanol leaves extract, BSHXSB = *B. salviifolia* hexane stem-bark extract, BSCHSB = *B. salviifolia* chloroform stem-bark extract, BSEASB = *B. salviifolia* ethyl acetate stem-bark extract, BSMESB = *B. salviifolia* methanol stem-bark extract; Asc. acid = Ascorbic acid which served as positive control. The experiments were carried out in triplicates and each value is expressed as mean±standard deviation (*n*=3).

Figure 1. The percentage inhibition of DPPH radical by various leaves extracts of *B. salviifolia* at various concentrations.



BSHXLS = *B. salviifolia* hexane leaves extract, BSCHLS = *B. salviifolia* chloroform leaves extract, BSEALS = *B. salviifolia* ethyl acetate leaves extract, BSMELS = *B. salviifolia* methanolic leaves extract; Asc. acid = Ascorbic acid which served as positive control. The experiments were carried out in triplicates. Each value is expressed as mean±standard deviation (*n*=3).

Figure 2. The percentage inhibition of DPPH radical by various stem-bark extracts of *B. salviifolia* at various concentrations.



BSHXSB = *B. salviifolia* hexane stem-bark extract, BSCHSB = *B. salviifolia* chloroform stem-bark extract, BSEASB = *B. salviifolia* ethyl acetate stem-bark extract, BSMESB = *B. salviifolia* methanolic stem-bark extract; Asc**.** acid = Ascorbic acid which served as positive control. The experiments were carried out in triplicates. Each value is expressed as mean±standard deviation (*n*=3).