

## Phytochemical analysis, total flavonoid, total phenolic contents and ferric reducing power of extracts from leaves and stem bark of *Buddleja salviifolia*

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### Abstract

Chloroform, ethyl acetate and methanolic extracts from leaves and stem bark of *Buddleja salviifolia* were prepared. The phytochemical profiles, total flavonoid contents (TFC), total phenolic contents (TPC) and ferric reducing power of these extracts were studied. The presence of alkaloids, tannins, phlobatannins, saponins, sterols, flavonoids, coumarins, phenolics, terpenoids, quinones and reducing sugars were identified from these extracts. The TFC of these extracts was found to be in the range of 11.36±0.98 to 30.76±1.15 mg QE/g DW, respectively. The TPC of these extracts was found to be in the range of 175.00±0.41 to 465.27±2.65 mg GAE/g DW, respectively. Additionally, these extracts showed a weak to moderate ferric reducing power compared to ascorbic acid. *B. salviifolia* finds therapeutic applications in traditional medicine. Further studies are required to commercialize products from this plant.

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## 1. Introduction

*Buddleja salviifolia* belongs to the Scrophulariaceae family of the *Buddleja* genus (Van Wyk and Van Wyk, 1997; Ream, 2006; Tank *et al.*, 2006; Van Laere *et al.*, 2011). *B. salviifolia* is also known by other names such as sailehout, sagewood, wildsaile and *lelothoane* (Van Wyk and Van Wyk, 1997; Kose *et al.*, 2015). *B. salviifolia* is a semi-evergreen shrub (Ream, 2006) or trees of 3-8 m height (Drummond, 1981) and is widely distributed in the Southern Africa (Palmer, 1997). *B. salviifolia* finds therapeutic applications in the traditional medicine in the African continent. Particularly, the leaves of *B. salviifolia* possess most of the therapeutic potentials and have been used to treat TB, herpes, syphilis, cervical cancer, complications associated with pregnancy, coughs, colic, constipation, neurodegenerative diseases, eye infections and to relieve nausea (Pendota *et al.*, 2013; Kose *et al.*, 2015). The antimicrobial and DPPH radical scavenging activities of various extracts from leaves and stem bark of *B. salviifolia* have previously been reported (Matamane *et al.*, 2019; Pillai *et al.*, 2019). The present study aimed to analyse the phytochemical profiles, total flavonoid contents (TFC), total phenolic contents (TPC) and ferric

reducing power of chloroform, ethyl acetate and methanolic extracts from leaves and stem bark of *B. salviifolia* species collected from the Kingdom of Lesotho, Southern Africa. This is the first report of this kind from the *B. salviifolia* and particularly, the species gathered from the Kingdom of Lesotho.

## 2. Materials and methods

### 2.1 Chemicals and Reagents

AR grades (99.5%) of chloroform, ethyl acetate, methanol, benzene, quercetin, gallic acid and trichloroacetic acid were all purchased from Sigma Aldrich. Benedict's reagent, sodium hydroxide, hydrochloric acid and sulphuric acid were purchased from Minema Chemicals. Potassium ferricyanide, ferric chloride, sodium dihydrogen phosphate, magnesium powder, ascorbic acid, trisodium phosphate and potassium mercuric iodide were purchased from Prestige Laboratory Supplies.

### 2.2 Plant materials

The leaves and stem bark of *B. salviifolia* were collected in August 2018 at the National University of Lesotho, Roma campus. Voucher specimens for leaves

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(Matamane/BSLS/2018) and stem bark (Matamane/BSSB/2018) were preserved in the Organic Research Laboratory, Department of Chemistry and Chemical Technology, National University of Lesotho, Roma Campus, Maseru, Lesotho.

### 2.3 Processing of materials

The leaves were allowed to air-dry at room temperature for two weeks and then ground into powder (523.86 g) 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 for two weeks and then ground into powder (760.46 g) using the same blender.

### 2.4 Preparation of extracts

A mass of 98.88 g of powdered leaves was macerated with 500 mL of chloroform for three days with occasional shaking. The resulting solution was filtered using a vacuum filter (ATB, Model: 284965-H, Power: 230 v 3.0 A, 1320/min, 50 Hz) and the extract was concentrated *in vacuo*. The concentrated extract was then transferred to a pre-weighted clean and dry beaker. The procedure was replicated two times. Finally, the sample was refluxed with chloroform for 10 hrs and the resulting solution was concentrated *in vacuo*. A mass of 7.48 g of combined chloroform extract was obtained. Similarly, 8.13 and 14.61 g of ethyl acetate and methanolic extracts were obtained, respectively from 100.23 and 175.45 g of powdered leaves. Using the similar extraction procedure, 1.07, 2.18 and 8.86 g of chloroform, ethyl acetate and methanolic extracts were obtained, respectively from 200.09, 200.01, 202.53 and 157.82 g of powdered stem bark.

### 2.5 Phytochemical analysis

Chloroform, ethyl acetate and methanolic extracts thus obtained from the leaves and stem bark of *B. salviifolia* were screened for their phytochemicals such as alkaloids, tannins, phlobatannins, saponins, sterols, flavonoids, coumarins, phenolics, terpenoids, quinones and reducing sugars using the methods described in the literature (Trease and Evans, 1984; Trease and Evans, 2002; Tiwari et al., 2011; Saeed et al., 2012; Soni and Sheetal, 2013; Nwaoguikpe et al., 2014; Pandey and Tripathi, 2014; Uddin et al., 2014).

#### 2.5.1 Detection of alkaloids

A mass of 5.0 mg of each extract was dissolved in 2.0 mL of distilled water separately and then three drops of Wagner's reagent was added. The formation of a reddish-brown precipitate indicates the presence of

alkaloids.

#### 2.5.2 Detection of phenolics

A mass of 0.5 g of each extract was dissolved in 1.0 mL of distilled water separately and was heated in a water bath at 95°C for 30 mins. The resulting mixture was filtered and 3.0 mL of 5% (w/v) of ferric chloride was added to the filtrate followed by the addition of 1.0 mL of 1% (w/v) potassium ferricyanide. A formation of green colouration indicates the presence of simple phenols and the blue colouration indicates the presence of polyphenols.

#### 2.5.3 Detection terpenoids

A volume of 0.5 mL of distilled was added to 0.25 g of each extract separately and then 1.0 mL of chloroform was added with vigorous shaking and swirling. A formation of reddish-brown colouration on the interface upon the addition of 3.0 mL of concentrated sulphuric acid to the mixture indicates the presence of terpenoids.

#### 2.5.4 Detection of flavonoids

A mass of 0.50 g of each extract was dissolved in 2 mL of methanol and heated in a water bath separately. A small amount of magnesium powder was added to the mixture and few drops of concentrated hydrochloric acid were added thereafter. A formation of dark brown colouration which gradually shades to deep red or pink colouration indicates the presence of flavonoids.

#### 2.5.5 Detection of sterols

A mass of 0.3 g of each extract was dissolved in 2.0 mL of chloroform separately and then filtered off. A volume of 1.0 mL of concentrated sulphuric acid was added to each filtrate. A formation of two phases of which the chloroform phase turns red indicates the presence of sterols.

#### 2.5.6 Detection of saponins

A volume of 2.0 mL of distilled water was added to 0.1 g of each extract separately and the contents were agitated in a test tube for 15 mins. A formation of a 1 cm layer of foam indicates the presence of saponins.

#### 2.5.7. Detection of tannins

A mass of 0.2 g of each extract was dissolved in 2.0 mL of distilled water separately and heated in a water bath at 95°C. Upon filtration, few drops of concentrated sulphuric acid were added to each filtrate followed by the addition of a few drops of 5% (w/v) ferric chloride. A deposition of blue, black, green or blue-green precipitate indicates the presence of tannins.

### 2.5.8 Detection of phlobatannins

A mass of 0.1 g of each extract was boiled in 3.0 mL of 1% aqueous hydrochloric acid separately. A formation of a red precipitate indicates the presence of phlobatannins.

### 2.5.9 Detection of quinones

A mass of 0.25 g of each extract was dissolved in 0.5 mL of distilled water separately and then 1.5 mL of concentrated hydrochloric acid was added to it. A deposition of green or white precipitate indicates the presence of quinones.

### 2.5.10 Detection of reducing sugars

A mass of 0.1 g of each extract was dissolved in 2.5 mL of distilled water separately. A volume of 2.0 mL of the extract solution was mixed with 2.0 mL of Benedict's solution. Each of these mixed contents was heated in a boiling water bath at 95°C for 5 mins or until there was an observable colour change. A formation of brick-red colouration indicates the presence of reducing sugars.

### 2.5.11. Detection of coumarins

A mass of 0.3 g of each extract was dissolved in 1.0 mL of distilled water separately and then 1.0 mL of 10% (w/v) sodium hydroxide and 1.0 mL of chloroform were added. A formation of yellow colour indicates the presence of coumarins.

## 2.6. Determination of total flavonoid contents (TFC)

The TFC of each of these six extracts was evaluated separately using the aluminum chloride calorimetric method as described in the literature (Joshi *et al.*, 2019). The results were calculated based on a calibration curve plotted using quercetin. A stock solution of quercetin was prepared by dissolving 25 mg of quercetin in 1.0 mL of methanol and serial dilutions of 25, 20, 15, 10 and 5 µg/mL were prepared from the stock solution. A solution of 10 mL of each extract in methanol at a concentration of 1000 µg/mL was prepared separately. Aliquots of 3.0 mL of each extract or quercetin were mixed separately with 2% (w/v) aluminum chloride in clean and oven-dried test tubes. The experiment was performed in triplicates and the contents were incubated at room temperature for 15 mins. The absorbance was measured at 420 nm using MRS Spectro UV-11 spectrophotometer. The total flavonoid contents of each of these extracts were estimated from the quercetin calibration plot and are expressed as milligrams of quercetin equivalents per gram of extract dry weight (mg QE/g DW).

## 2.7 Determination of phenolic contents (TPC)

The TPC of each of these six extracts was determined separately using Folin-Ciocalteu assay (Kokate, 1994) with slight modifications. The results were calculated based on a calibration curve plotted using gallic acid. Briefly, 1000 µg/mL of stock solution of gallic acid was prepared by dissolving 10.0 mg of gallic acid in 10 mL of methanol. Two-fold serial dilutions of 1000 to 15.625 µg/mL were prepared from the stock solution. A solution of 10 mL of each extract in methanol at a concentration of 1000 µg/mL was prepared separately. Aliquots of 2.0 mL of each extract or gallic acid solution were mixed separately with 1.0 mL of 1% (v/v) Folin-Ciocalteu reagent in clean and oven-dried test tubes. The reaction mixture was allowed to stand for 4 mins and then 1.0 mL of 0.7M sodium carbonate was added. The content was shaken vigorously for 15 s then incubated in the dark for 25 mins at room temperature with occasional agitation. The experiment was performed in triplicates. The absorbance of the reaction mixture was measured at 765 nm using MRS Spectro UV-11 spectrophotometer. The total phenolic content of each extract was estimated from the gallic acid calibration plot and are expressed as milligrams of gallic acid equivalents per gram of extract dry weight (mg GAE/g DW).

## 2.8 Evaluation of ferric reducing power

The ferric reducing power of these six extracts was determined using a method described in literature (Fejes *et al.*, 2000) with slight modification. The ability of an extract to transform Fe (III) to Fe (II) has been termed as its ferric reducing power (Fejes *et al.*, 2000; Saeed *et al.*, 2012). The formation of Prussian blue colour indicates the transformation and the measure of absorbance at 700 nm indicates its ferric reducing power (Saeed *et al.*, 2012). Briefly, a stock solution of 0.2 mg of each extract in 1 mL of methanol was prepared, separately. Two-fold serial dilutions of 200 to 0 µg/mL were prepared. The 0 µg/mL solution served as negative control while ascorbic acid served as a positive control. Aliquots of 2.0 mL of each extract at various concentrations were mixed with 2.0 mL of 0.2M phosphate buffer (pH 6.6), 2.0 mL of 0.01% (w/v) potassium ferricyanide solution and the mixture was separately incubated at 50°C. A volume of 2.0 mL of 0.1% (w/v) trichloroacetic acid was added to the mixture and the content was centrifuged at 3000 rpm for 10 mins. The upper layer was collected thereafter and aliquots of 2.0 mL of the supernatant liquid were mixed further with 0.4 mL of 0.1% (w/v) freshly prepared ferric chloride solution and 2.0 mL of distilled water. The absorbance (optical density) of the content was measured at 700 nm using a spectrophotometer (MRS Spectro UV-

11) after allowing the reaction to occur for 10 mins. Ferric reducing power is a measure of absorbance in a reaction and the higher absorbance indicates the higher the reducing power of an extract or pure compound (Saeed *et al.*, 2012).

### 2.9 Statistical analysis

All determinations were performed in triplicates ( $n = 3$ ) and the results are expressed as mean $\pm$ SD. Data analysis was performed using SPSS 17.0 application software by means of two-way analysis of variance (ANOVA). The means were considered statistically significant when  $p \leq 0.05$ .

## 3. Results and discussion

A total of six extracts viz. *B. salviifolia* chloroform leaves extract (1), *B. salviifolia* ethyl acetate leaves extract (2), *B. salviifolia* methanolic leaves extract (3), *B. salviifolia* chloroform stem bark extract (4), *B. salviifolia* ethyl acetate stem bark extract (5) and *B. salviifolia* methanolic stem bark extract (6) were prepared. These six extracts (1-6) were subjected to phytochemical analysis and the results are summarized in Table 1. Extract 1 showed the presence of phenolics, flavonoids, sterols and coumarins. Extract 2 showed the presence of alkaloids, phenolics, terpenoids, flavonoids, sterols, tannins, phlobatannins, quinones and reducing sugars. Extract 3 exhibited the presence of phenolics, terpenoids, flavonoids, sterols, saponins, tannins, quinones, reducing sugars and coumarins. Extract 4 showed the presence of alkaloids, phenolics, terpenoids, flavonoids and sterols. Extract 5 exhibited the presence of alkaloids, phenolics, terpenoids, sterols, tannins, phlobatannins, quinones, reducing sugars and coumarins. Extract 6 showed the presence of phenolics, terpenoids, flavonoids, sterols, saponins, tannins and reducing sugars (Table 1).

Table 1. Phytochemical screening of various extracts from leaves and stem bark of *Buddleja salviifolia*

Phytoconstituent	Extracts					
	1	2	3	4	5	6
Alkaloids	-	+	-	+	+	-
Phenolics	+	+	+	+	+	+
Terpenoids	-	+	+	+	+	+
Flavonoids	+	+	+	+	-	+
Sterols	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
Tannins	-	+	+	-	+	+
Phlobatannins	-	+	-	-	+	-
Quinones	-	+	+	-	+	-
Reducing sugars	-	+	+	-	+	+
Coumarins	+	-	+	-	+	-

(+) and (-) signs indicate the presence and absence of the phytoconstituent, respectively.

The plant-derived secondary metabolites such as flavonoids and phenolics have ideal structural characteristics for free radical scavenging activity (Amarowicz *et al.*, 2004). They have an aromatic ring bearing one or more hydroxyl groups (Tungmunnithum *et al.*, 2018). These hydroxyl groups have the ability to donate hydrogen atoms to deactivate free radicals (Amarowicz *et al.*, 2004). In phenolics, the number of hydroxyl groups contributes directly to the potency of antioxidant activity (Bendary *et al.*, 2013). In flavonoids, however, the potency of antioxidant activity depends not only on the number of free hydroxyl groups but also on their position on the flavonoid skeleton (Panche *et al.*, 2016). TFC and TPC of 1-6 are summarized in Table 2. Quercetin and gallic acid served as standards for TFC and TPC, respectively and their calibration curves are shown in Figures 1 and 2, respectively. The TFC of 1-6 was found to be  $30.76 \pm 1.15$ ,  $24.32 \pm 0.62$ ,  $19.93 \pm 0.24$ ,  $16.92 \pm 0.70$ ,  $13.99 \pm 0.66$  and  $11.36 \pm 0.98$  mg QE/g DW, respectively. This result indicated that 1 exhibited higher TFC relative to the other two leaf extracts viz. 2 and 3. Similarly, 4 showed higher TFC relative to the other two stem bark extracts viz. 5 and 6. Additionally, this result also revealed the fact that chloroform was found to be suitable for the extraction of flavonoids from plant materials (Pandey and Tripathi, 2014). The TPC of 1-6 was found to be  $175.00 \pm 0.41$ ,  $392.77 \pm 0.44$ ,  $465.27 \pm 2.65$ ,  $182.44 \pm 0.51$ ,  $318.02 \pm 1.01$  and  $281.93 \pm 0.12$  mg GAE/g DW, respectively. Extract 3 exhibited a higher TPC relative to the other two (1 and 2) leaf extracts. Similarly, 5 exhibited a higher TPC relative

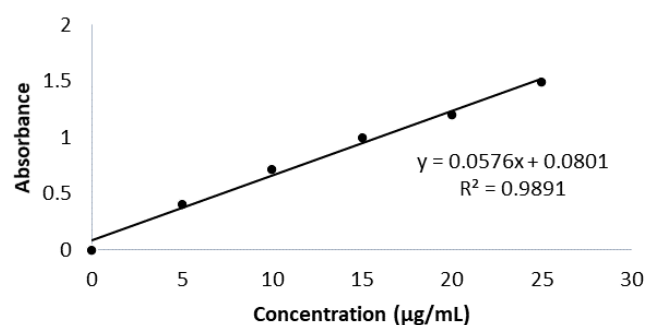


Figure 1. The calibration curve of quercetin (used to estimate TFC)

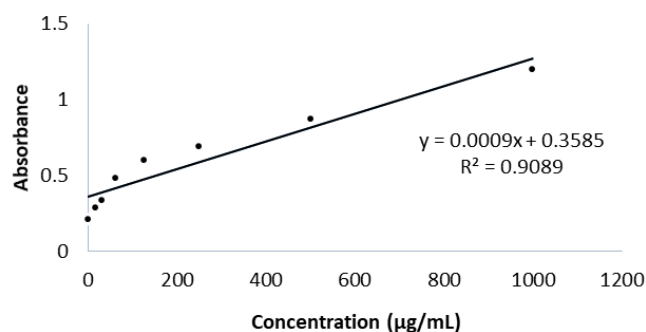


Figure 2. The calibration curve of gallic acid (used to estimate TPC)

Table 2. Total flavonoid contents (TFC) and total phenolic contents (TPC) of various extracts from leaves and stem bark of *Buddleja salviifolia*

Extracts	TFC (mg QE/g)	TPC (mg GAE/g)	Ferric reducing power/concentration of extracts in $\mu\text{g/mL}$				
			12.5	25	50	100	200
1	30.76 $\pm$ 1.15	175.00 $\pm$ 0.41	0.179 $\pm$ 0.03	0.184 $\pm$ 0.00	0.186 $\pm$ 0.01	0.199 $\pm$ 0.01	0.217 $\pm$ 0.05
2	24.32 $\pm$ 0.62	392.77 $\pm$ 0.44	0.207 $\pm$ 0.01	0.212 $\pm$ 0.08	0.244 $\pm$ 0.01	0.292 $\pm$ 0.05	0.387 $\pm$ 0.02
3	19.93 $\pm$ 0.24	465.27 $\pm$ 2.65	0.200 $\pm$ 0.01	0.270 $\pm$ 0.00	0.346 $\pm$ 0.07	0.491 $\pm$ 0.09	0.656 $\pm$ 0.00
4	16.92 $\pm$ 0.70	182.44 $\pm$ 0.51	0.189 $\pm$ 0.03	0.192 $\pm$ 0.01	0.217 $\pm$ 0.04	0.244 $\pm$ 0.00	0.278 $\pm$ 0.00
5	13.99 $\pm$ 0.66	318.02 $\pm$ 1.01	0.204 $\pm$ 0.06	0.222 $\pm$ 0.07	0.266 $\pm$ 0.00	0.313 $\pm$ 0.02	0.407 $\pm$ 0.01
6	11.36 $\pm$ 0.98	281.93 $\pm$ 0.12	0.229 $\pm$ 0.01	0.285 $\pm$ 0.02	0.291 $\pm$ 0.00	0.362 $\pm$ 0.00	0.512 $\pm$ 0.02
Asc. acid	N/A	N/A	0.437 $\pm$ 0.09	0.554 $\pm$ 0.01	0.663 $\pm$ 0.11	0.812 $\pm$ 0.01	0.992 $\pm$ 0.09

Values are expressed as mean $\pm$ SD of triplicates ( $n = 3$ ). TFC: Total flavonoid contents, TPC: Total phenolic contents. Asc. Acid: ascorbic acid, N/A: Not applicable.

to the other two (4 and 6) stem bark extracts.

The ability of flavonoids and phenolics to transform a  $\text{Fe}^{3+}$  (ferricyanide complex) to a  $\text{Fe}^{2+}$  (ferrous cyanide complex) acts as a potential indicator for the antioxidant activity (Meir *et al.*, 1995). In other words, the ferric salt is reduced to ferrous salt and the flavonoids and phenolics are oxidized. The absorbance (optical density) for this oxidation process could be measured which is a measure of the antioxidant capacity of flavonoids and phenolics. The ferric reducing power of 1-6 and the positive control (ascorbic acid) have a linear relation with concentrations (refer to Table 2 and Figures 1 and 2). The ferric reducing power of 1-3, 4-6 and ascorbic acid were found to be in the ranges of 0.179 $\pm$ 0.03-0.656 $\pm$ 0.00, 0.189 $\pm$ 0.03-0.512 $\pm$ 0.02 and 0.437 $\pm$ 0.09-0.992 $\pm$ 0.09, respectively (refer to Table 2). Among leaf extracts, 3 showed the highest ferric reducing power of 0.656 $\pm$ 0.00 at a concentration of 200  $\mu\text{g/mL}$  followed by 2 (0.387 $\pm$ 0.02) and 1 (0.217 $\pm$ 0.05), respectively. Among stem bark extracts, 6 exhibited the highest ferric reducing power of 0.512 $\pm$ 0.02 at a concentration of 200  $\mu\text{g/mL}$  followed by 5 (0.407 $\pm$ 0.01) and 4 (0.278 $\pm$ 0.00).

However, all three leaf extracts (1-3) and all three stem bark extracts (4-6) showed lower ferric reducing power compared to a positive control (refer to Table 2 and Figure 3).

This is the first report on the preliminary phytochemical screening of various extracts from *B. salviifolia*.

However, terpenoids have previously been reported from other species of the *Buddleja* genus (Houghton *et al.*, 2003). In a previous report, the TFC of dichloromethane/methanol (1:1) and water extracts from whole plants of *B. salviifolia* have been reported as 23.95 $\pm$ 0.11 and 12.11 $\pm$ 0.26 mg QE/g DW (milligrams of quercetin equivalents per gram of dry sample), respectively (Adewusi *et al.*, 2011). Similarly, the TPC of these two extracts has been reported as 169.66 $\pm$ 0.33 and 77.92 $\pm$ 0.91 mg TAE/g DW (milligrams of tannic acid equivalents per gram of dry sample), respectively (Adewusi *et al.*, 2011). In the present study, the TFC and TPC of various extracts from leaves and stem bark of *B. salviifolia* were found to be in the range of 11.36 $\pm$ 0.98 to

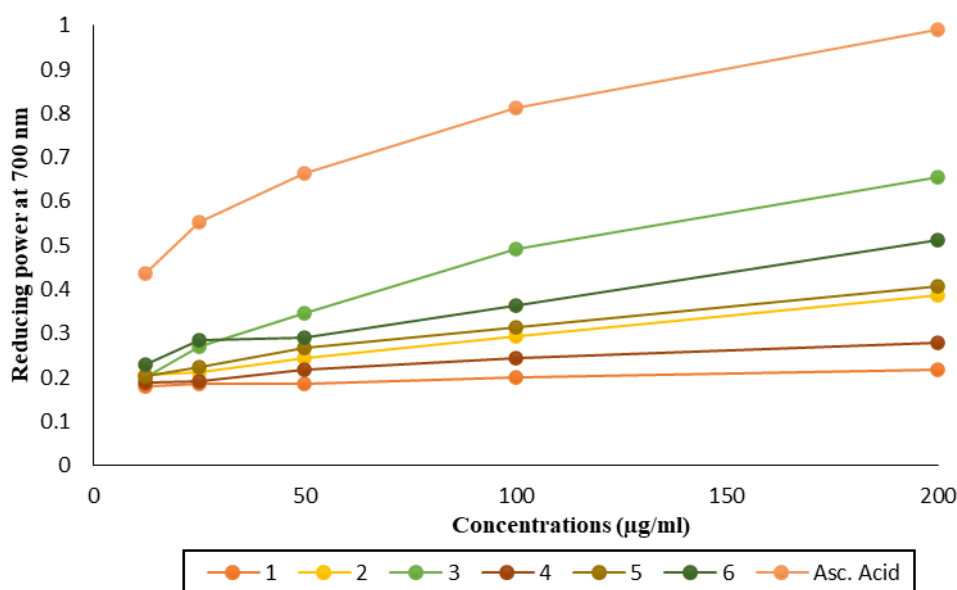


Figure 3. Reducing power of various extracts from leaves and stem bark of *B. salviifolia* at various concentrations. Asc. acid = ascorbic acid.

30.76±1.15 mg QE/g DW and 175.00±0.41 to 465.27±2.65 mg GAE/g DW, respectively. The discrepancy in the TFC and TPC in our study and the previous report may be due to the polarity of different solvents and plant parts used for the extraction of active components. Extracts 2, 3, 5 and 6 showed significant ferric reducing power due to the presence of tannins (refer to Table 1). Tannins have been reported to have ferric reducing power (Okuda, 2005). Various extracts from *B. salviifolia* have previously been reported to have DPPH radical scavenging activity (Matamane et al. 2019), which could be due to the presence of phytochemicals such as polyphenols, phenolics and flavonoids. These phytochemicals have been reported as important classes of natural antioxidants (Array et al., 2019; Rodrigues et al., 2019).

#### 4. Conclusion

The phytochemical profiles, TFC, TPC and ferric reducing power of chloroform, ethyl acetate and methanolic extracts from leaves and stem bark of *B. salviifolia* were studied. The presence of alkaloids, tannins, phlobatannins, saponins, sterols, flavonoids, coumarins, phenolics, terpenoids, quinones and reducing sugars was identified from these extracts. The TFC of these extracts was found to be in the range of 11.36±0.98 to 30.76±1.15 mg QE/g DW, respectively. The TPC of these extracts was found to be in the range of 175.00±0.41 to 465.27±2.65 mg GAE/g DW, respectively. Additionally, these extracts showed a weak to moderate ferric reducing power compared to ascorbic acid. Further studies are required to commercialize products from this plant.

#### Conflict of interests

The authors declare no conflicts of interest.

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