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Research Article

Antioxidant Activity of Extracts from *Bidens pilosa*-A Medicinal Plant from the Kingdom of Lesotho

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Abstract: *Bidens pilosa* finds therapeutic applications in the traditional medicine. In the present study, we aimed to evaluate the antioxidant activity of various extracts obtained from the leaves and stem-bark of *B. pilosa* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing power assay. We also aimed to determine the total phenolic contents (TPCs) by Folin-Ciocalteu colorimetric method and total flavonoid contents (TFCs) by the aluminium chloride colorimetric method. Various solvent extracts were obtained from the leaves and stem-bark of *B. pilosa* by maceration and hot solvent extraction techniques. The extracts from leaves and stem-bark exhibited radical scavenging activity in the ranges of 4.40 ± 1.02-81.18 ± 1.14% and 5.92 ± 2.16-79.34 ± 8.43%, respectively at concentrations of 200-3,000 µg/mL. The positive control, ascorbic acid exhibited radical scavenging activity in a range of 55.17 ± 1.89-88.12 ± 1.31% at the same concentrations. Additionally, the methanol extracts from both leaves and stem-bark were found to be the most potent with IC_{50} values of 848.54 and < 200 µg/mL, respectively. The positive control, ascorbic acid exhibited an IC_{50} value < 200 µg/mL. Furthermore, the extracts from the leaves and stem-bark exhibited ferric reducing power in the ranges of 0.095 ± 0.017-1.175 ± 0.084 and 0.093 ± 0.006-1.100 ± 0.065, respectively at concentrations of 5-100 µg/mL. The positive control, ascorbic acid exhibited a ferric reducing power in the range of 0.326 ± 0.00-1.213 ± 0.078 at concentrations of 5-100 µg/mL. In addition, the TPCs of extracts from the leaves and stem-bark were determined to be in the ranges of 9.03 ± 2.34-179.31 ± 0.96 and 10.93 ± 1.04-154.04 ± 2.15 mg GAE/g of DW of extract, respectively. Similarly, the TFCs of extracts from the leaves and stem-bark were determined to be in the ranges 1.28 ± 0.05-29.33 ± 4.19 and 1.89 ± 0.58-27.75 ± 2.67 mg QE/g of DW of extract, respectively. From this study, we concluded that various extracts obtained from *B. pilosa* showed a moderate to strong radical scavenging and ferric reducing power and possessed a significant amount of TPCs and TFCs.

***Keywords*:** *Bidens pilosa*, Asteraceae, DPPH assay, IC_{50} value, ferric reducing power, TPCs, TFCs

1. Introduction

Bidens pilosa belongs to the *Bidens genus of* Asteraceae family, which consists of 280 species.[1,2] *B. pilosa* is known by vernacular names such as blackjack, bohome, farmer's friends and Spanish needle.[2] *B. pilosa* is native to the

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Americas but it has widely been distributed throughout the world including Africa, Australia, South America and the Pacific Islands as introduced species.[2] *B. pilosa* has been used in the treatment of hepatitis, laryngitis, conjunctivitis, abscesses, headache, urinary infections and digestive disorder and it has been exhibited varieties of biological and pharmacological activities, which include antimalarial, antihyperglycaemic, antileukemic, antitumor, antibacterial, antimicrobial, anti-inflammatory and antioxidant activities.[2-13] Practically, all parts of *B. pilosa* are exhibited therapeutic applications.[13] Therefore, the whole plant materials have been used in the traditional medicine for the preparation of decoctions or infusions for internal consumption.[13] Various classes of phytochemicals have been reported from *B. pilosa* which include phenolics, flavonoids, polyphenols, terpenoids, saponins, alkaloids, phenylpropanoids[6,14], sesquiterpene lactones[14,15], lipids, benzenoids[13,16-20], flavonoid glycosides[21,22], friedelanes and lupeol derivatives, flavonoids and polyacetylenes[23,24] and their conjugated compounds[14,24], di-O-caffeoylquinic acids and polyacetylene glucoside compounds.[24] Approximately, 300 different compounds have been isolated and reported from various parts of *B. pilosa*[14,24,25] and the pharmaceutical properties of this plant have been due to the presence of these bioactive secondary metabolites. For example, the presence of flavonoid and polyacetylene compounds in *B. pilosa* have been responsible for antimalarial activity against *Plasmodium falciparum* and *Plasmodium berghei*.[25-27] The presence of acetylenic glucosides have been responsible for antihyperglycemic activity.[28] A large number of flavonoid compounds have also been exerted anti-colorectal cancer activity.[22,29-31] Flavonoids such as isoquercitrin, quercetin and catechin[29-31] have been exhibited anticancer potential by inhibiting cell proliferation.[22,29-32] In addition, flavonoid compounds have been reported as effective anti-inflammatory agents[6,14,15] and compounds such as sesquiterpene lactones and polyacetylenes have been inhibited the growth of pathogenic microorganisms.[6,14,15] Furthermore, compounds such as flavonoids, phenolics, polyphenols and lactones have been exhibited free radical scavenging[6,12,14,22] and antioxidant potentials[33,34].

In a previous study, a methanol crude extract has been obtained from leaves of *B. pilosa* collected in Mizoram, India by maceration technique.[6] In another study, acetone, methanol and aqueous extracts have been obtained from the leaves of *B. pilosa* collected in Alice, South Africa by maceration technique.[13] Additionally, aqueous and methanol extracts have been obtained from the flowers of *B. pilosa* gathered in Taichung, Taiwan by placing the samples in a sonicator bath at ambient temperature.[24] Furthermore, 70% ethanol crude extract has been obtained from the whole plant of *B. pilosa* collected in Fujian province of China by hot solvent extraction at reflux condition[22] and six pure flavonoid compounds have been isolated from ethyl acetate fraction obtained from this 70% ethanol crude extract.[22] In another study, the essential oils have been obtained by hydrodistillation using a Clevenger-type apparatus from fresh leaves of *B. pilosa* collected in Moutourwa, Cameroon.[12] All these solvent extracts obtained from the above maceration, hot solvent extraction and hydrodistillation extraction techniques have been evaluated for their radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[6,12,13,22,24] The results obtained from these studies have already been reported[6,12,13,22,24] and are summarized in this article under the section of Results and Discussion (refer to Results and Discussion section). Additionally, the total phenolic contents **(**TPCs) and total flavonoid contents (TFCs) of these extracts have also been reported previously[6,12,13,22,24] and are summarized in this article under the section of Results and Discussion (refer to Results and Discussion section) and are listed in Table 3.

Our literature search showed that maceration, hot solvent extraction at reflux condition and hot extraction by hydrodistillation techniques have been utilized to obtain various extracts from various parts of *B. pilosa*.[6,12,13,22,24] The advantages of maceration technique are that it is simple, easy to set-up and it has widely been used to extract a wide range of compounds. Since the extraction is performed under mild condition at room temperature, the extracted compounds are usually obtained without decomposition. One of the major disadvantages of this maceration technique is that it has poor efficiency to extract compounds since this is a mild extraction technique and carried out under room temperature. Additionally, this maceration technique takes considerably longer time to complete the extraction process. On the other hand, hot solvent extraction techniques are more efficient in extracting compounds and consume relatively lesser time for extraction. However, the disadvantage of the hot solvent extraction techniques has been that some compounds of interest may undergo decomposition under reflux conditions due to thermo-lability nature of compounds at reflux temperature. In the present study, we used maceration technique first to extract as much as compounds possible without decomposition and the samples were recovered and then subjected to hot solvent extraction at reflux condition to extract the remaining compounds. In other words, more efficient and optimal extraction of compounds from the plant materials were achieved with the combination of maceration and hot solvent extraction techniques. Our literature search showed that methanol, acetone and water extracts from leaves[6,13], aqueous and methanol extracts from flowers[24], 70%

ethanolic extract from whole plants[22] and essential oils from leaves[12] have been evaluated for antioxidant activity[6,12,13,22]. Additionally, we also noticed that extracts such as hexane, chloroform and ethyl acetate from various parts of *B. pilosa* have not been explored for antioxidant activity, particularly, the species gathered from the Kingdom of Lesotho. Therefore, in the present study, we aimed to evaluate the antioxidant activity of various extracts obtained from leaves and stem-bark of *B. pilosa* gathered from the Kingdom of Lesotho by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing power assay. We also aimed to determine the TPCs and TFCs of these extracts by Folin-Ciocalteu colorimetric method and aluminium chloride colorimetric method, respectively. The results obtained from the above studies are summarized in the present article.

2. Materials and methods

2.1 *Plant materials*

Fresh plant materials of *B. pilosa* were gathered in January 2022 in a garden located at Hata-butle village of Roma, Maseru district, Lesotho, Southern Africa. Dr. Seleteng-Kose, Department of Biology, National University of Lesotho (NUL), identified the plant materials. Samples of leaves (Mahase/BPLS/2022) and stem-bark (Mahase/BPSB/2022) were deposited at the Organic Chemistry Research Lab, NUL.

2.2 *Processing of plant materials*

The air-dried plant materials were ground into powder using a Waring Blender (Model HGB2WT93). A mass of 1.323 and 4.378 kg of powder were obtained from leaves and stem-bark, respectively.

2.3 *Preparation of plant extracts*

A mass of 255.027 g of powdered leaves was macerated with hexane for 24 h at room temperature of 28-30 °C. The solvent was removed by vacuum distillation and the hexane crude extract thus obtained was kept aside. The leaf powder was recovered from the above maceration process and was extracted again with hexane under reflux condition for 24 h. Following the usual procedure and after removal of solvent, 3.754 g of combined crude hexane extract was obtained. The aforementioned procedure was used to obtain 5.957, 4.900, 10.262, and 20. 612 g of chloroform, ethyl acetate, acetone, and methanol crude extracts, respectively from 251.841, 250.783, 250.952 and 250.145 g of powdered leaves. Similarly, 2.992, 2.855, 1.349, 2.855 and 4.194 g of hexane, chloroform, ethyl acetate, acetone and methanol crude extracts were obtained, respectively from 251.025, 250.127, 250.781, 250.923, 250.552 g of powdered stem-bark.

2.4 *Solvents, reagents and chemicals*

Analytical reagents (AR) grades of chemicals such as gallic acid, tris-(hydroxymethyl)aminomethane and Folin-Ciocalteu reagent, AR grades of solvents *viz*. methanol, ethyl acetate, chlorofrom and hexane, were all obtained from Sigma-Aldrich. Sodium hydroxide, disodium hydrogen carbonate and sodium dihydrogen phosphate were purchased from Minema Ltd. 2,2-Diphenyl-1-picrylhydrazyl and ascorbic acid were obtained from Prestige Laboratory Supplies. Aluminium chloride and sodium carbonate were obtained from Associated Chemical Enterprises. Ferric chloride and trichloroacetic acid were purchased from BDH Chemicals Ltd. Potassium ferricyanide, quercetin and sodium carbonate were purchased respectively from Holpro Analytics Pty Ltd, Acros Organics and Radchem Laboratory Supplies. Sodium phosphate and sodium nitrite were obtained from Saarchem Pty Ltd.

2.5 *Radical scavenging assay and IC_{50} values*

The evaluation of antioxidant activity and the determination of IC_{50} values of various extracts obtained from the leaves and stem-bark of *B. pilosa* were achieved using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay by a method described in the literature.[35,36] The preparation of stock solutions of each extract and positive control, ascorbic acid (3.0 mg of each extract or ascorbic acid in 1.0 mL of 50% methanol (v/v), further dilutions from each of

these stock solutions (3,000, 2,000, 1,500, 1,000, 800, 500 and 200 µg/mL) and the preparation of negative control (50% methanol blank solution, v/v) were as per literature.[35] An oxidant solution was prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol. A test solution was prepared by mixing 0.1 mL of each extract solution or positive control with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCL buffer (pH 7.4). The absorbance of the mixture was taken at 517 nm after 30 min incubation. The experiments were carried out in triplicates and the average value of three experiments were used to calculate the percentage inhibition of radical scavenging ability by the equation given below.

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

A_{test} = Absorbance of extract solution or positive control. A_{cont} = Absorbance of negative control. The IC_{50} value, the concentration in µg/mL of an extract or pure compound that inhibits the formation of DPPH radical by fifty percent, was determined by plotting extract concentrations *versus* the percentage inhibition of DPPH radical.[35,37]

2.6 *Ferric reducing power assay*

The evaluation of the ferric reducing power of various extracts obtained from *B. pilosa* was carried out using a method described in literature.[35] The preparation of stock solutions of each extract and positive control, ascorbic acid (0.2 mg of each extract or ascorbic acid in 1.0 mL of methanol), further dilutions from each of these stock solutions (5, 10, 20, 40, 80 and 100 µg/mL) and the preparation of negative control (50% methanol blank solution, v/v) were as per literature.[35] A reaction mixture consisted of 2.0 mL of 0.2 M phosphate buffer (pH 6.6), 2.0 mL of 0.01% potassium ferricyanide solution and 2.0 mL of each solution of extract or ascorbic acid. A volume of 2.0 mL of 0.1% trichloroacetic acid was added to the reaction mixture after 20 min incubation at 50 °C. The supernatant liquid was collected separately after a centrifugation of the mixture at 3,000 rpm for 10 min. An aliquot of each supernatant liquid was mixed separately with 2.0 mL of freshly prepared 0.1% ferric chloride solution and then 2.0 mL of distilled water was added. The absorbance of the content was measured at 700 nm after allowing the reaction mixture to stand for 10 min. Ferric reducing power is the ability of an extract or pure compound to reduce Fe (III) to Fe (II).[35] The ability of this ferric reducing power is indicated by the formation of Prussian blue coloration and is measured at 700 nm. All experiments were conducted in triplicates and the results were reported as the average value of three experiments. A higher value of absorbance represents higher ferric reducing power of an extract or pure compound and *vice versa*.[35]

2.7 *Total phenolic contents (TPCs)*

Folin-Ciocalteu colorimetric method was employed to determine TPCs of various extracts obtained from the leaves and stem-bark of *B. pilosa* as per literature.[35,38] Gallic acid served as standard to get a calibration curve (Figure 1). The preparation of a test solution of each extract (10 mL at a concentration of 1,000 µg/mL in 50% methanol v/v), a stock solution of gallic acid (1,000 µg of gallic acid in 1.0 mL of 50% methanol, v/v), further dilutions from the stock solution of gallic acid (750, 500, 250, 150, 100 and 25 µg/mL), the preparation of negative control (50% methanol blank solution, v/v) and rest of the experimental procedure were as per details outlined in the literature.[35] Briefly, an aliquot of 0.3 mL of each extract solution or gallic acid solution was mixed separately with 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent followed by addition of 5 mL of 7.5% sodium carbonate. The reaction mixture was incubated at room temperature in the dark for a period of 2 h. The absorbance of each of this mixture was measured at 510 nm. All experiments were carried out in triplicates and the results were reported as the average value of three determinations. Gallic acid calibration plot was employed to estimate the total phenolic contents of each extract and is expressed as mg GAE/g DW. The calibration curve of gallic acid ($y = 0.0022x + 0.1483$; $R^2 = 0.9559$) in a concentration range of 100-600 µg/mL is given below (refer to Figure 1).

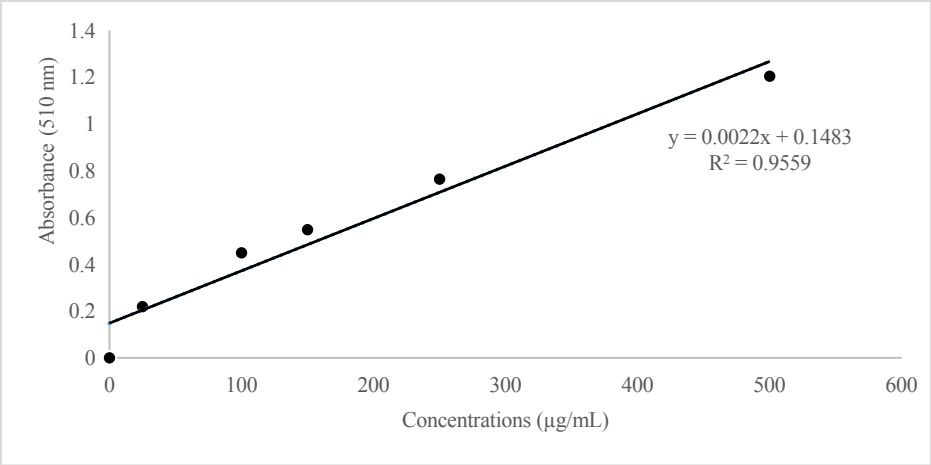

Figure 1. The calibration curve of gallic acid

2.8 *Total flavonoid contents (TFCs)*

Aluminum chloride colorimetric method was employed to determine the TFCs of various extracts obtained from the leaves and stem-bark of *B. pilosa* as per literature.[35,39] Quercetin served as standard to get a calibration curve (Figure 2). The preparation of a test solution of each extract (10 mL at a concentration of 1,000 µg/mL in 50% methanol, v/v), a stock solution of quercetin (100 µg in 1.0 mL of, 50% methanol, v/v), further dilutions from this quercetin stock solution (30, 25, 20, 15, 10 and 5 µg/mL), the preparation of negative control (50% methanol blank solution, v/v) and the rest experimental procedure were as per the details outlined in the literature.[35] Briefly, an aliquot of 0.3 mL of each extract solution or quercetin solution was mixed separately with 0.3 mL of 5% sodium nitrite followed by addition of 0.3 mL of 10% aluminum chloride after 5 min interval. The reaction mixture was allowed to stand for a minute and a volume of 2 mL of 1.0 M sodium hydroxide and 6 mL of deionized water was added. The absorbance of each mixture was measured at 510 nm. All experiments were carried out in triplicates and the results were reported as the average value of three determinations. The quercetin calibration curve was employed to determine the total flavonoid contents of each extract and is expressed as mg QE/g DW. The calibration curve of quercetin ($y = 0.0507x + 0.1445$; $R^2 = 0.9713$) in a concentration range of 5-30 µg/mL is given below (refer to Figure 2).

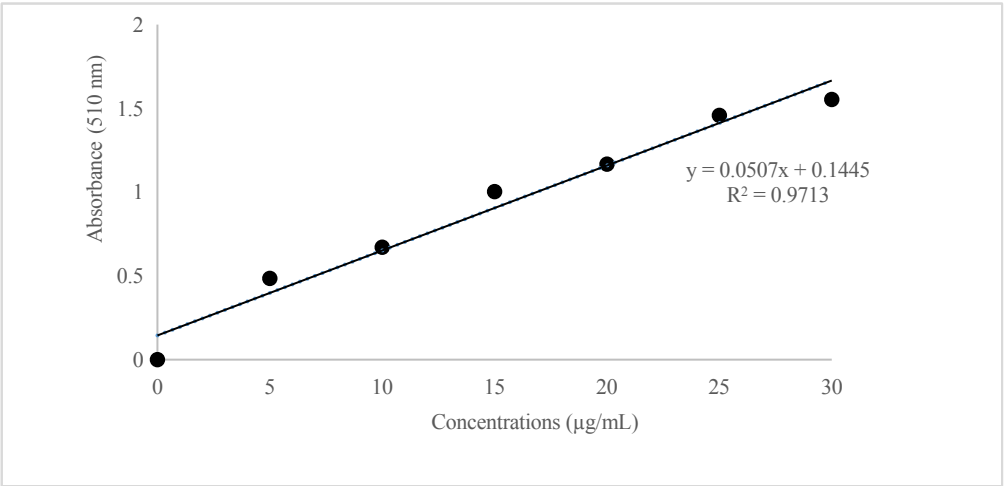

Figure 2. The calibration curve of quercetin.

2.9 *Statistical analysis*

Statistical analysis was performed using SPSS software version 28.0.0.0 for DPPH radical scavenging assay and SPSS v23.0 two-way analysis of variance (ANOVA) was used for ferric reducing power assay. When $p \leq 0.05$, the differences were statistically significant.

3. Results and discussion

The extracts labelled as **E1-E5** were respectively hexane, chloroform, ethyl acetate, acetone, methanol and water extracts from leaves of *B. pilosa* and the extracts labelled as **E6-E10** were respectively hexane, chloroform, ethyl acetate, acetone, methanol and water extracts from stem-bark of *B. pilosa*. The radical scavenging activity of these ten extracts **(E1-E10)** and positive control, ascorbic acid is summarized in Table 1. The extracts from leaves showed the following order of radical scavenging activity: **E5** > **E4** > **E3** > **E2** > **E1** (Table 1). The positive control showed a radical scavenging of 88.12 ± 1.31% at a concentration of 3,000 µg/mL. Among the extracts from leaves, **E5** showed a highest scavenging activity followed by **E4** at a concentration of 3,000 µg/mL. Both **E5** and **E4** showed low scavenging activity at low concentrations, they showed significant scavenging activity at higher concentrations. Extract **E5** showed slightly lower scavenging activity compared to positive control at higher concentrations. Extract **E3** exhibited slightly higher scavenging activity than **E2** at a concentration of 3,000 µg/mL and **E1** showed lowest scavenging activity at the same concentration at 3,000 µg/mL (Table 1). The stem-bark extracts exhibited the following order of radical scavenging activity: **E9** > **E10** > **E8** > **E7** > **E6** (Table 1). Extract, **E9** showed the highest scavenging activity at a concentration of 3,000 µg/mL. The scavenging activity of **E10** was comparable to **E9** at a concentration of 3,000 µg/mL. Similarly, **E8** and **E7** showed comparable scavenging activity to each other at a concentration of 3,000 µg/mL. Extract **E6** exhibited the lowest scavenging ability from at a concentration at 3,000 µg/mL (Table 1). Although all extracts exhibited lower scavenging, **E4**, **E5**, **E5** and **E10** showed significantly higher scavenging activity and **E2**, **E3**, **E7** and **E8** showed a moderate scavenging activity at a concentration of 3,000 µg/mL. Extracts **E1** and **E6** showed poorest scavenging among leaf and stem-bark extracts, respectively at a concentration of 3,000 µg/mL. For ease of comparison, the scavenging activity of **E1-E10** and ascorbic acid are shown in the bar diagrams (Figure 3 and Figure 4).

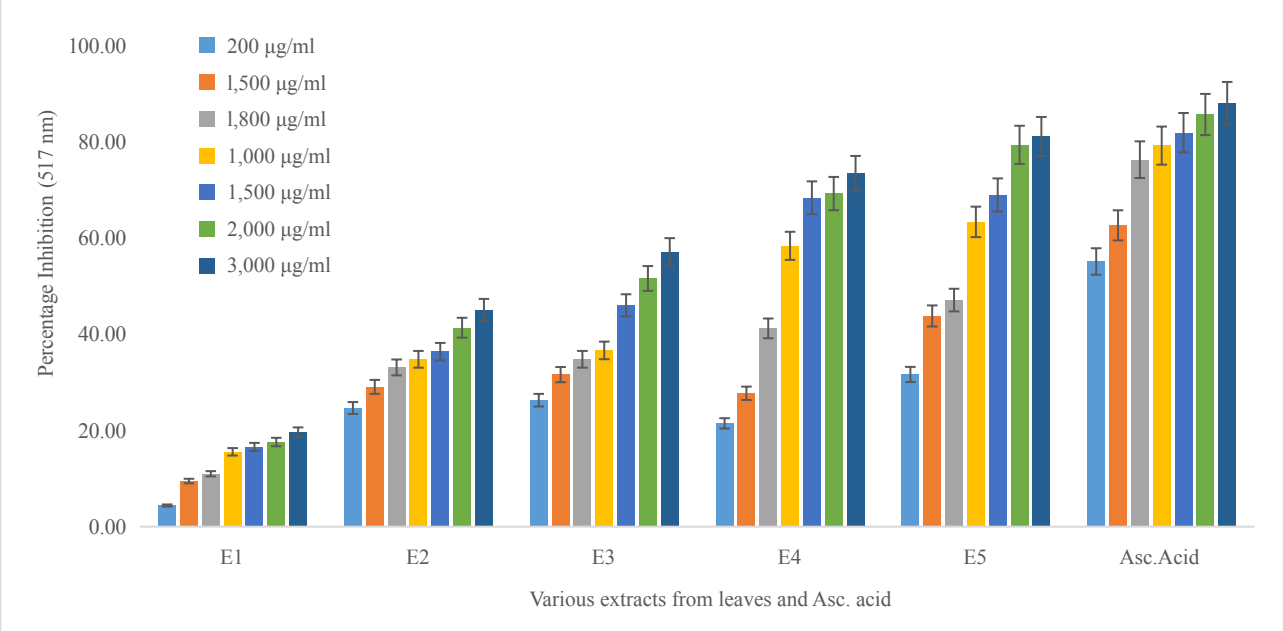

Figure 3. Radical scavenging of various leaves extracts from *B. pilosa* and ascorbic acid.
E1 = Hexane extract from leaves, **E2** = chloroform extract from leaves, **E3** = ethyl acetate extract from leaves, **E4** = acetone extract from leaves, **E5** = methanol extract from leaves.

Table 1. DPPH radical scavenging activity, determination of IC_{50} values, TPCs and TFCs of various extracts from *B. pilosa*.

Extracts	Concentrations (µg/mL)/Inhibition (%)							IC_{50} (µg/mL)	TPCs (mg GAE/g DW)	TFCs (mg QE/g DW)
	200	500	800	1,000	1,500	2,000	3,000			
E1	4.40 ± 1.02^{d}	9.49 ± 1.15^{c}	11.00 ± 3.22^{e}	15.57 ± 2.88^{f}	16.60 ± 1.69^{e}	17.61 ± 2.73^{b}	19.67 ± 3.71^{d}	> 3,000	9.03 ± 2.34	1.28 ± 0.05
E2	24.69 ± 2.54^{e}	29.08 ± 4.53^{d}	33.13 ± 0.65^{a}	34.82 ± 1.02^{c}	36.41 ± 2.22^{d}	41.39 ± 2.31^{c}	45.13 ± 1.11^{b}	> 3,000	48.40 ± 1.34	15.22 ± 0.86
E3	26.31 ± 6.57^{f}	31.63 ± 3.97^{d}	34.54 ± 1.32^{c}	36.67 ± 4.13^{d}	46.05 ± 2.06^{c}	51.64 ± 3.79^{c}	57.17 ± 0.41^{a}	1,628.66	46.92 ± 3.03	10.75 ± 2.12
E4	21.49 ± 2.89^{d}	27.75 ± 1.10^{b}	41.25 ± 0.91^{a}	58.43 ± 7.23^{e}	68.42 ± 2.51^{e}	69.30 ± 3.14^{d}	73.46 ± 4.00^{e}	969.70	100.12 ± 1.10	28.07 ± 2.32
E5	31.66 ± 2.24^{d}	43.84 ± 0.29^{a}	47.14 ± 1.12^{c}	63.41 ± 2.50^{c}	69.01 ± 5.22^{e}	79.44 ± 4.26^{d}	81.18 ± 1.14^{c}	848.54	179.31 ± 0.96	29.33 ± 4.19
E6	5.92 ± 2.16^{d}	7.88 ± 1.24^{b}	11.40 ± 3.47^{e}	18.04 ± 2.14^{c}	19.44 ± 2.54^{c}	23.12 ± 5.84^{e}	24.67 ± 2.13^{c}	> 3,000	10.93 ± 1.04	1.89 ± 0.58
E7	19.41 ± 1.62^{b}	35.89 ± 2.09^{d}	38.43 ± 5.15^{f}	38.67 ± 3.04^{d}	45.31 ± 3.45^{e}	48.15 ± 1.03^{a}	50.81 ± 2.91^{d}	2,076.84	38.88 ± 0.72	16.45 ± 0.44
E8	20.91 ± 1.33^{c}	30.48 ± 1.41^{c}	35.70 ± 0.58^{b}	40.58 ± 2.18^{e}	49.90 ± 5.70^{f}	52.71 ± 1.85^{b}	54.68 ± 1.83^{b}	1,503.01	51.01 ± 4.23	13.23 ± 1.08
E9	26.51 ± 1.75^{c}	32.85 ± 1.88^{c}	34.59 ± 3.24^{d}	48.76 ± 1.15^{a}	67.19 ± 4.44^{c}	76.76 ± 0.81^{a}	79.23 ± 6.15^{e}	1,025.43	96.27 ± 0.29	24.31 ± 1.17
E10	52.58 ± 1.66^{d}	56.36 ± 2.97^{d}	59.94 ± 3.07^{e}	60.21 ± 1.30^{b}	72.53 ± 0.75^{c}	79.34 ± 6.98^{f}	79.07 ± 8.43^{f}	< 200	154.04 ± 2.15	27.75 ± 2.67
Asc. acid	55.17 ± 1.89^{c}	62.69 ± 0.94^{a}	76.35 ± 0.11^{b}	79.28 ± 0.44^{a}	81.97 ± 2.04^{b}	85.75 ± 0.87^{a}	88.12 ± 1.31^{b}	< 200	N/A	N/A

E1 = Hexane extract from leaves, **E2** = chloroform extract from leaves, **E3** = ethyl acetate extract from leaves, **E4** = acetone extract from leaves, **E5** = methanol extract from leaves, **E6** = hexane extract from stem-bark, **E7** = chloroform extract from stem-bark, **E8** = ethyl acetate extract from stem-bark, **E9** = acetone extract from stem-bark, **E10** = methanol extract from stem-bark. N/A = Not applicable. Values with different superscript letters are statistically different within column.

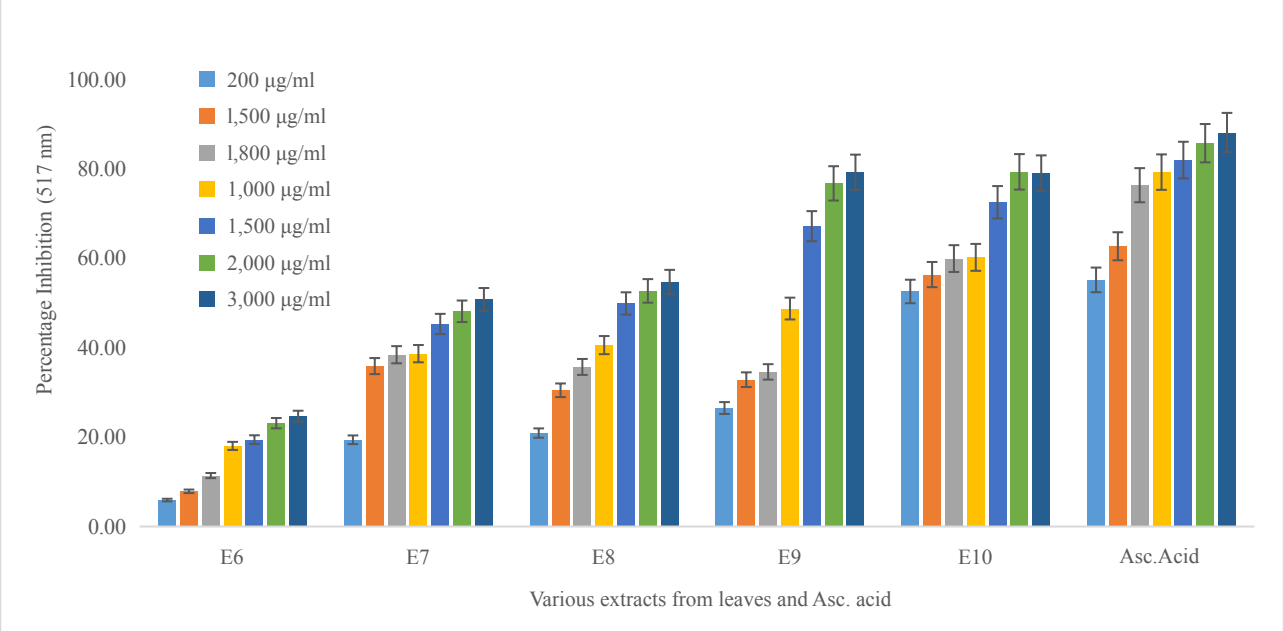

Figure 4. Radical scavenging of various stem-bark extracts from *B. pilosa* and ascorbic acid.
E6 = hexane extract from stem-bark, **E7** = chloroform extract from stem-bark, **E8** = ethyl acetate extract from stem-bark, **E9** = acetone extract from stem-bark, **E10** = methanol extract from stem-bark.

The IC_{50} values of **E1-E10** and ascorbic acid are summarized in Table 2. Ascorbic acid exhibited an IC_{50} value of < 200 µg/mL. The IC_{50} values of **E1-E10** were determined to be > 3,000, > 3,000, 1,628.66, 969.70, 848.54, > 3,000, 2,076.84, 1,503.01, 1,025.43 and < 200 µg/mL, respectively. Extract **E5** was found to be the most potent among the extracts from leaves with an IC_{50} value of 848.54 µg/mL followed by **E4** with an IC_{50} value of 969.70 µg/mL. Similarly, **E10** was found to be the most potent among the extracts from stem-bark with an IC_{50} value of < 200 µg/mL followed by **E9** with an IC_{50} value of 1025.43 µg/mL. Extracts **E8, E7** and **E6** showed relatively higher scavenging activity with IC_{50} values of 1,503.01, 2,076.84 and > 3,000 µg/mL, respectively. Extract **E6** showed poorest scavenging among all ten extracts with an IC_{50} value of > 3,000 µg/mL. Overall, **E5** and **E10** were identified as the most potent extracts and their IC_{50} values were determined to be 848.54 and < 200 µg/mL, respectively (Table 1).

In previous studies, the antioxidant activities of various extracts from *B. pilosa* collected at various locations have been reported. For example, a methanolic leaf extract of *B. pilosa* collected in Mizoram, India has been evaluated for antioxidant activity in DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays and the IC_{50} of values were determined to be 80.45 and 171.6 µg/mL, respectively in these assays.[6] Additionally, acetone, methanol and aqueous extracts obtained from the flowers of *B. pilosa* collected in South Africa have been evaluated for DPPH and ABTS radical scavenging activity.[13] The acetone, methanol and aqueous extracts have been exhibited 95.7, 94.2 and 91.7%, respectively, activity in the DPPH assay at a concentration of 1,000 µg/mL.[13] Similarly, acetone methanol and aqueous extracts have also been exhibited 98.8, 99.1 and 95.7% respectively, activity in the ABTS radical scavenging assay at a concentration of 1,000 µg/mL.[13] This result revealed that all three extracts showed relatively higher scavenging activity in the ABTS assay compared to DPPH assay at a concentration of 1,000 µg/mL.[13] In another study, aqueous and methanol extracts have been obtained from the flowers of *B. pilosa* collected in Taiwan.[24] These two extracts have been evaluated for their DPPH radical scavenging activity and the IC_{50} values of aqueous and methanol crude extracts have been determined to be 1,045.4 ± 63.6 and 207.3 ± 43.5 µg/mL, respectively, in the DPPH assay.[24] Furthermore, a 70% ethanol crude extract has been obtained from the whole plant of *B. pilosa* collected in China.[22] Purification of ethyl acetate fraction obtained from this 70% ethanol crude extract led to isolate pure compounds such as quercetin, vitexin, astragalin, isoquercitrin, 5,6,7,4'-tetramethoxylflavone and 5,3',4'-trihydroxy-3,7-dimethoxylflavone.[22] The IC_{50} values of these pure compounds have been determined to be in the ranges of 15.2 ± 0.4-99.8 ± 2.3 µmol/L in the DPPH assay and in the ranges of 21.3 ± 1.7-212.3 ± 1.6 µmol/L in the ABTS assay,

respectively.[22] However, vitexin and 5,6,7,4'-tetramethoxylflavone did not exhibit any activity in the DPPH and ABTS assays.[22] In another study, a methanolic leaf extract from *B. pilosa* collected in Cameroon has been evaluated for DPPH radical scavenging activity and it exhibited 77.40% radical scavenging activity at a concentration of 20 mg/L in the DPPH assay.[12]

Table 2. Ferric reducing power of extracts from *B. pilosa*

Extracts	Concentrations (µg/mL)					
	5	10	20	40	80	100
E1	0.102 ± 0.015^{a}	0.168 ± 0.042^{d}	0.222 ± 0.007^{a}	0.269 ± 0.051^{e}	0.376 ± 0.041^{c}	0.464 ± 0.021^{b}
E2	0.095 ± 0.017^{b}	0.153 ± 0.031^{c}	0.280 ± 0.048^{d}	0.369 ± 0.003^{a}	0.510 ± 0.023^{b}	0.603 ± 0.009^{a}
E3	0.118 ± 0.075^{e}	0.197 ± 0.000^{a}	0.215 ± 0.011^{a}	0.301 ± 0.056^{e}	0.525 ± 0.044^{c}	0.723 ± 0.034^{c}
E4	0.213 ± 0.073^{e}	0.352 ± 0.062^{e}	0.394 ± 0.053^{e}	0.470 ± 0.015^{a}	0.953 ± 0.093^{f}	1.175 ± 0.084^{f}
E5	0.287 ± 0.041^{d}	0.376 ± 0.017^{b}	0.418 ± 0.004^{a}	0.530 ± 0.074^{e}	0.986 ± 0.065^{e}	1.096 ± 0.103^{f}
E6	0.093 ± 0.006a	0.118 ± 0.052^{e}	0.186 ± 0.043^{c}	0.306 ± 0.023^{b}	0.389 ± 0.098^{f}	0.444 ± 0.032^{c}
E7	0.113 ± 0.048^{d}	0.167 ± 0.021^{b}	0.265 ± 0.015^{a}	0.317 ± 0.043^{c}	0.440 ± 0.035^{e}	0.490 ± 0.048^{d}
E8	0.210 ± 0.004^{a}	0.276 ± 0.091^{f}	0.377 ± 0.024^{b}	0.400 ± 0.091^{f}	0.634 ± 0.017^{b}	0.712 ± 0.000^{a}
E9	0.345 ± 0.087^{f}	0.421 ± 0.056^{e}	0.608 ± 0.106^{f}	0.788 ± 0.054^{e}	1.006 ± 0.043^{e}	1.118 ± 0.074^{e}
E10	0.296 ± 0.045^{d}	0.398 ± 0.042^{d}	0.515 ± 0.034^{c}	0.706 ± 0.063^{e}	0.975 ± 0.031^{c}	1.100 ± 0.065^{e}
Asc. Acid	0.326 ± 0.005^{a}	0.485 ± 0.040^{c}	0.518 ± 0.057^{e}	0.597 ± 0.046^{d}	0.905 ± 0.071^{e}	1.213 ± 0.078^{f}

Refer to footnotes of Table 1.

The ferric reducing power of all ten extracts (**E1-E10**) and the positive control, ascorbic acid are given in Table 2. The range of ferric reducing power of **E1-E5** was found to be 0.095 ± 0.017 to 1.175 ± 0.084 and for ascorbic acid it was found to be 0.326 ± 0.005 to 1.213 ± 0.078. The leaf extracts showed the following order of ferric reducing power: **E4** > **E5** > **E3** > **E2** > **E1**. Therefore, **E4** showed the highest reducing potential among the extracts from leaves and its reducing power was found to be 1.175 ± 0.084 at a concentration of 100 µg/mL. The reducing power of **E6-E10** was found to be in the range 0.093 ± 0.006 to 1.100 ± 0.065. The stem-bark extracts showed the following order of ferric reducing power: **E9** > **E10** > **E8** > **E6** > **E5**. Therefore, **E9** showed the highest ferric reducing potential among the extracts from stem-bark with its reducing power of 1.118 ± 0.074 at a concentration of 100 µg/mL. For comparison and clarity purposes, the dose-response curves for ferric reducing power of extracts from leaves and stem-bark are given in Figures 5 and 6, respectively.

In a previous study, the ferric reducing antioxidant power (FRAP) of methanolic extracts obtained from leaves, stems and roots of *B. pilosa* have been reported[20] and these extracts showed FRAP values of 15.3 ± 1.17, 10.1 ± 0.87 and 73.2 ± 6.5 µg/mL, respectively.[40] Similarly, the FRAP of acetone, methanol and water extracts obtained from the leaves of *B. pilosa* were determined to be 2,431.93 ± 143.95, 561.68 ± 10.57 and 35.70 ± 0.08, respectively.[13] The FRAP of positive controls such as ascorbic acid, BHT, catechin and quercetin have been determined to be 1,632.1 ± 16.95, 63.46 ± 2.49, 972.02 ± 0.61 and 3,107.29 ± 31.28, respectively.[13] In another study, the FRAP of aqueous leaf extract, methanolic leaf extract and hexane, ethyl acetate and *n*-butanol fractions obtained from methanolic leaf extract have also

been evaluated against trolox.[24] The ethyl acetate fraction has been shown strongest FRAP and showed higher FRAP than trolox at high concentration of 1,000 µg/mL.[24] In addition, essential oil obtained on day 1 from fresh leaves of *B. pilosa* and the essential oils stored in 5, 10 and 15 days, respectively were evaluated for their ferric reducing powers at various concentrations.[12] In general, the ferric reducing powers of these essential oils have been increased with increasing concentrations and decreased with increasing storage periods.[12] The essential oil prepared on day 1 exhibited ferric reducing power of 14.88 ± 0.52-59.55 ± 0.98 at a concentration range of 1-20 mg/L.[12] However, the essential oils stored in 5, 10 and 15 days exhibited ferric reducing power of 7.77 ± 0.73-41.65 ± 0.31, 4.93 ± 0.73-32.06 ± 0.81 and 1.78 ± 0.52-19.14 ± 1.27 respectively at a concentration range of 1-20 mg/L.[12]

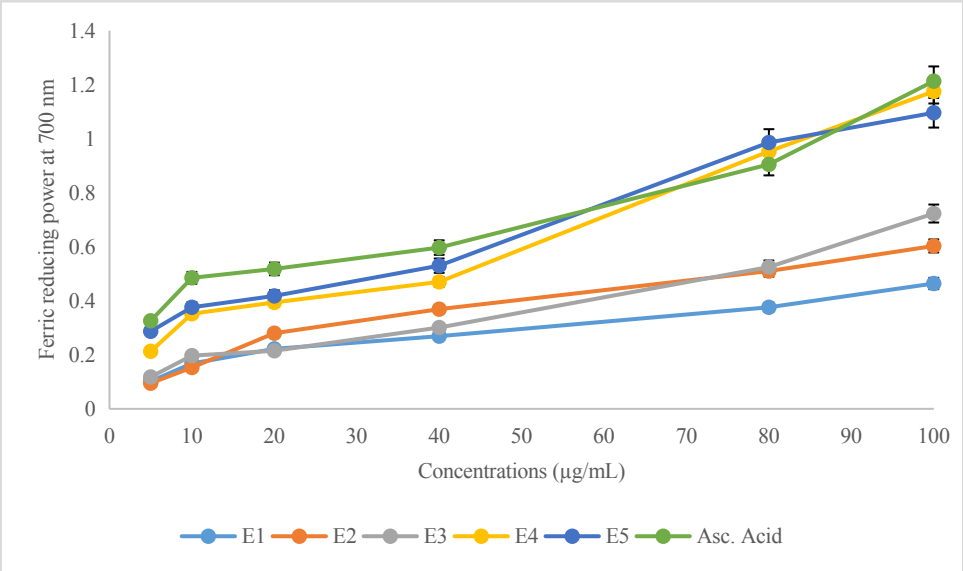

Figure 5. Dose-response curve for various extracts (**E1-E5**) obtained from leaves of *B. pilosa* for their ferric reducing power. **E1** = Hexane extract from leaves, **E2** = chloroform extract from leaves, **E3** = ethyl acetate extract from leaves, **E4** = acetone extract from leaves, **E5** = methanol extract from leaves.

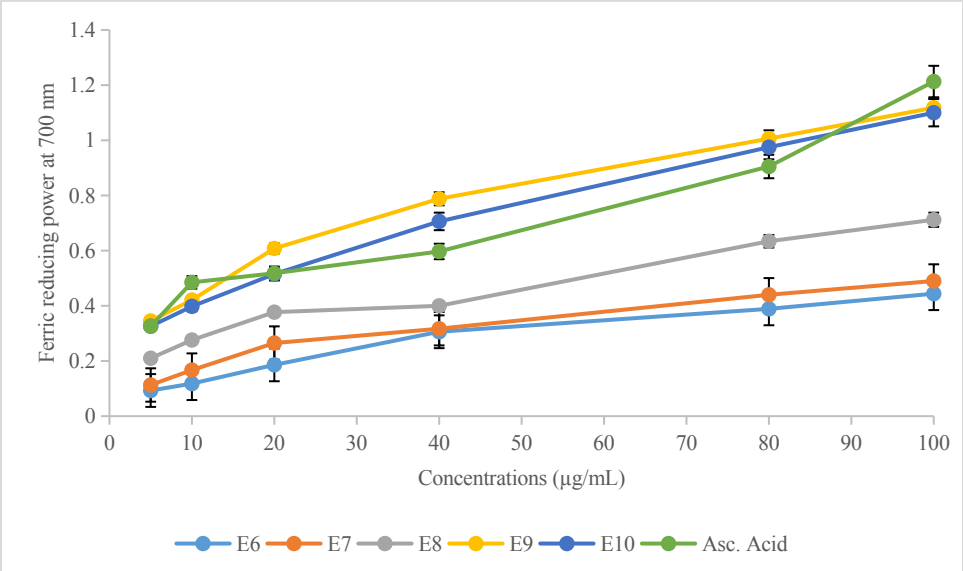

Figure 6. Dose-response curve for various extracts (**E6-E10**) obtained from stem-bark of *B. pilosa* for their ferric reducing power. **E6** = hexane extract from stem-bark, **E7** = chloroform extract from stem-bark, **E8** = ethyl acetate extract from stem-bark, **E9** = acetone extract from stem-bark, **E10** = methanol extract from stem-bark.

Table 3. TPCs and TFCs of various extracts obtained from various parts of *B. pilosa* collected in four different countries.[6,13,24]

S. No.	Name of the location and country in which the plants were collected	Plant parts used	Solvents used for extraction/ fractionation	TPCs (mg STD/g DW)	TFCs (mg QE/g DW)	References
1	Alice, South Africa	Leaves	Aqueous	6.09 ± 0.28	0.77 ± 0.01	[13]
2	Alice, South Africa	Leaves	Acetone	32.53 ± 1.10	2.01 ± 0.07	[13]
3	Alice, South Africa	Leaves	Methanol	27.08 ± 2.90	0.93 ± 0.02	[13]
4	Taichung, Taiwan	Flowers	Aqueous	105.05 ± 3.73	172.11 ± 2.14	[24]
5	Taichung, Taiwan	Flowers	Methanol	538.10 ± 0.96	235.06 ± 3.46	[24]
6	Taichung, Taiwan	Flowers	Hexane fraction	51.71 ± 4.76	79.82 ± 2.45	[24]
7	Taichung, Taiwan	Flowers	Ethyl acetate fraction	752.15 ± 7.53	469.10 ± 0.97	[24]
8	Taichung, Taiwan	Flowers	*n*-Butanol fraction	236.02 ± 1.10	363.19 ± 5.07	[24]
9	Taichung, Taiwan	Flowers	Aqueous fraction	25.12 ± 0.43	108.08 ± 8.49	[24]
10	Mizoram, India	Leaves	Methanol	0.072	0.1233	[6]
11	Roma, Kingdom of Lesotho	Leaves	Hexane	9.03 ± 2.34	1.28 ± 0.05	Present study
12	Roma, Kingdom of Lesotho	Leaves	Chloroform	48.40 ± 1.34	15.22 ± 0.86	Present study
13	Roma, Kingdom of Lesotho	Leaves	Ethyl acetate	46.92 ± 3.03	10.75 ± 2.12	Present study
14	Roma, Kingdom of Lesotho	Leaves	Acetone	100.12 ± 1.10	28.07 ± 2.32	Present study
15	Roma, Kingdom of Lesotho	Leaves	Methanol	179.31 ± 0.96	29.33 ± 4.19	Present study
16	Roma, Kingdom of Lesotho	Stem-bark	*n*-Hexane	10.93 ± 1.04	1.89 ± 0.58	Present study
17	Roma, Kingdom of Lesotho	Stem-bark	Chloroform	38.88 ± 0.72	16.45 ± 0.44	Present study
18	Roma, Kingdom of Lesotho	Stem-bark	Ethyl acetate	51.01 ± 4.23	13.23 ± 1.08	Present study
19	Roma, Kingdom of Lesotho	Stem-bark	Acetone	96.27 ± 0.29	24.31 ± 1.17	Present study
20	Roma, Kingdom of Lesotho	Stem-bark	Methanol	154.04 ± 2.15	27.75 ± 2.67	Present study

STD = Tannic acid for S. No. 1-3 and gallic acid for S. No. 4-20. QE = Quercetin for S. No. 1-20.

The TPCs leaf extracts (**E1-E5**) was determined to be 9.03 ± 2.34, 48.40 ± 1.34, 46.92 ± 3.03, 100.12 ± 1.10 and 179.31 ± 0.96 mg GAE/g DW, respectively. Therefore, **E5** possessed highest TPCs among the extracts from leaves followed by **E4**, **E2**, **E3** and **E1**. Similarly, the TPCs of stem-bark extracts (**E6-E10**) was found to be 10.93 ± 1.04, 38.88 ± 0.72, 51.01 ± 4.23, 96.27 ± 0.29 and 154.04 ± 2.15 mg GAE/g DW, respectively. This result showed that **E10** has highest TPCs among stem-bark extracts followed by **E9**, **E8**, **E7** and **E6**. Similarly, the TFCs of leaf extracts (**E1-E6**) was found to be 1.28 ± 0.05, 15.22 ± 0.86, 10.75 ± 2.12, 28.07 ± 2.32 and 29.33 ± 4.19 mg QE/g DW, respectively. Therefore, **E5** possessed highest TFCs among the extracts from leaves followed by extracts **E4**, **E2**, **E3** and **E1**. The

TFCs of stem-bark extracts (**E6-E10**) was determined to be 1.89 ± 0.58, 16.45 ± 0.44, 13.23 ± 1.08, 24.31 ± 1.17 and 27.75 ± 2.67 mg QE/g DW, respectively. Therefore, **E10** possessed highest TFCs among the extracts from stem-bark followed by **E9**, **E7**, **E8** and **E6**.

The TPCs and TFCs of various extracts obtained from various parts of *B. pilosa* collected in four different countries are summarized in Table 3.[6,13,24] The TPCs of aqueous, acetone and methanol extracts obtained from the leaves of *B. pilosa* collected in Alice, South Africa have been determined to be 6.09 ± 0.28, 32.53 ± 1.10 and 27.08 ± 2.90 mg TAE/g DW, respectively.[13] In another study, the TPCs of an aqueous extract obtained from the fresh flowers of *B. pilosa* collected in Taichung, Taiwan has been determined to be 105.05 ± 3.73 mg GE/g DW.[24] On the other hand, the TPCs of a methanol crude extract obtained from the fresh flowers of *B. pilosa* and four of its fractions *viz.* hexane/water (1:1), ethyl acetate/water (1:1), *n*-butanol/water (1:1) and the remaining aqueous fraction have been determined to be 538.10 ± 0.96, 51.71 ± 4.76, 752.15 ± 7.53, 236.02 ± 1.18 and 25.12 ± 0.43 mg GE/g DW, respectively.[24] In the present study, the TPCs of various extracts from leaves and stem-bark of *B. pilosa* collected in Roma, the Kingdom of Lesotho were determined and compared with the previous studies[13,24] and are listed in Table 3. Similarly, the TFCs of aqueous, acetone and methanol extracts obtained from the leaves of *B. pilosa* collected in Alice, South Africa have been found to be 0.77 ± 0.01, 2.01 ± 0.07 and 0.93 ± 0.02 mg QE/g DW, respectively.[13] In another study, the TPCs of an aqueous extract obtained from the fresh flowers of *B. pilosa* collected in Taichung, Taiwan has been determined to be 172.11 ± 2.14 mg QE/g DW.[24] On the other hand, the TFCs of methanol crude extract obtained from the fresh flowers of *B. pilosa* and four of its fractions *viz.* hexane/water (1:1), ethyl acetate/water (1:1), *n*-butanol/water (1:1) and the remaining aqueous fraction have been determined to be 235.06 ± 3.46, 79.82 ± 2.45, 469.10 ± 0.97, 363.19 ± 5.07 and 108.08 ± 8.49 mg QE/g DW, respectively.[24] In another study, the TPCs of methanol crude extract obtained from the fresh leaves of *B. pilosa* collected in Mizoram, India has been determined to be 0.072 mg GE/g DW.[6] On the other hand, the TFCs of this methanol crude extract has been determined to be 0.1233 mg QE/g DW (Table 3).[6] In the present study, the TFCs of various extracts from both leaves and stem-bark of *B. pilosa* collected in Roma, the Kingdom of Lesotho were also determined and compared with the TFCs of previous studies[13,24] and are listed in Table 3.

From this study, we noticed that the methanol crude extract from leaves of *B. pilosa* collected in the Kingdom of Lesotho exhibited relatively lower TPCs and TFCs than the methanol crude extract and its fractions from the leaves of *B. pilosa* collected in Taiwan as reported in the literature (Table 3).[24] Additionally, it was also noticed that acetone and methanol crude extracts from leaves of *B. pilosa* collected in the Kingdom of Lesotho exhibited relatively higher TPCs and TFCs of than that of acetone and methanol crude extracts from leaves of *B. pilosa* collected in South Africa (Table 3).[13] Similarly, the methanol crude extract from leaves of *B. pilosa* collected in the Kingdom of Lesotho also exhibited relatively higher TPCs and TFCs of than methanol crude extract from leaves of *B. pilosa* collected in India. To summarize that in the present study, various extracts obtained from both leaves and stem-bark of *B. pilosa* collected in the Kingdom of Lesotho possessed significant TPCs and TFCs.

4. Conclusions

The antioxidant activity of various extracts from leaves and stem-bark of *B. pilosa* was investigated by DPPH radical scavenging assay and ferric reducing power assay. Acetone extract from leaves, methanol extract from leaves, acetone extract from stem-bark and methanol extract from stem-bark showed a high radical scavenging activity. The methanol extracts from both leaves and stem-bark were the most potent extracts in this DPPH assay. On the other hand, the acetone extract from leaves of *B. pilosa* exhibited a higher ferric reducing power compared to positive control ascorbic acid. Additionally, TPCs and TFCs of those extracts from leaves and stem-bark of *B. pilosa* were determined by Folin-Ciocalteu colorimetric method and aluminium chloride colorimetric method, respectively. Methanol extract from leaves showed highest TPCs followed by methanol extract from stem-bark, acetone extract from leaves and acetone extract from stem-bark. Similarly, the methanol extract from leaves showed highest TFCs followed by acetone extract from leaves, methanol extract from stem-bark and acetone extract from stem-bark. We concluded that various extracts obtained from *B. pilosa* showed a moderate to strong radical scavenging and ferric reducing power and possessed significant amount of TPCs and TFCs. Therefore, further studies on this plant will be useful to explore and justify the therapeutic applications of this plant.

Conflict of interest

The authors declare no competing financial interest.

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