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Antioxidant activity of extracts from *Xanthium strumarium* – A medicinal plant from the Kingdom of Lesotho

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ABSTRACT

Xanthium strumarium L. finds therapeutic applications in traditional medicines. The objective of the current study was to evaluate the antioxidant activity and to determine the total phenolic contents (TPCs) and total flavonoid contents (TFCs) of hexane, chloroform, ethyl acetate, acetone, methanol, and water extracts obtained from the leaves and stem bark of *X. strumarium*. Maceration and hot solvent extraction techniques were used to obtain various solvent extracts. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing power assays were used to evaluate the antioxidant activity. Folin-Ciocalteu colorimetric and aluminum chloride colorimetric methods were used to determine the TPCs and TFCs, respectively. The extracts from the leaves and stem bark exhibited radical scavenging activity in the ranges of 18.06 ± 0.3-185.67 ± 11.54% and 9.13 ± 0.54-84.18 ± 0.92%, respectively at a concentration range of 200-3000 µg/ml. The positive control, ascorbic acid, exhibited radical scavenging activity in a range of 56.64 ± 1.26-88.98 ± 0.31% at a concentration range of 200-3000 µg/ml. Additionally, the IC_{50} values of all these extracts were determined. The hexane and chloroform extracts from both leaves and stem bark and methanol leaf extract were found to be the most potent extracts with an IC_{50} value of < 200 µg/ml for each extract. The IC_{50} value of positive control, ascorbic acid was determined to be < 200 µg/ml. Furthermore, in the ferric-reducing power assay, ethyl acetate extract from both leaves and stem bark exhibited the highest ferric-reducing power of 0.996 ± 0.101 and 0.947 ± 0.018 at a concentration of 100 µg/ml. Moreover, the methanol extract from the leaves showed the highest TPCs of 133.41 ± 3.23 mg GAE/g of DW of extract followed by the methanol extract from stem bark and the acetone extract from the leaves with TPCs of 121.21 ± 3.14 and 118.01 ± 1.85 mg GAE/g of DW of extract, respectively. Similarly, the methanol extract from the leaves also showed the highest TFCs of 20.61 ± 1.81 mg QE/g of DW of extract followed by the methanol extract from stem bark with TFCs of 14.90 ± 1.18 mg QE/g of DW of extract. From this study, we concluded that various extracts obtained from the leaves and stem bark of *X. strumarium* exhibited a moderate-to-strong radical scavenging activity and ferric-reducing power and possessed a significant amount of TPCs and TFCs.

1. Introduction

Xanthium strumarium L. belongs to the *Xanthium* genus of the Asteraceae family. *X. strumarium* finds therapeutic applications, which include in the treatment of diabetes, headache, skin itch, arthritis gastric ulcer, nasal sinusitis, bacterial infections, and inflammatory illnesses such as rhinitis and rheumatoid arthritis (Aranjani et al., 2013; Fan et al., 2019; Kamboj & Saluja, 2010; Kumar & Rajkapoor, 2010; Patil et al., 2012). *X. strumarium* has been found to exhibit several biological and pharmacological activities, which include cytotoxicity, anticancer, antitrypanosomal, antiulcerogenic, larvicidal and repellent, antihelmintic, antiarthritic, antinociceptive, antiinflammatory, diuretic, antioxidant, antibacterial and antilipidemic activities

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(Kim et al., 2005; Lin et al., 2014; Ly et al., 2021; Scherer et al., 2009; Sharifi-Rad et al., 2015; Sharma et al., 2003; Singh et al., 2009; Sridharamurthy et al., 2011; Talakal et al., 1995; Tenguria, 2013). However, some studies showed that *X. strumarium* causes intoxication and is even fatal to humans and cattle (Masvingwe & Mavenyengwa, 1998; Turgut et al., 2005). For example, the seeds of *X. strumarium* cause multiple organ dysfunctions in humans, which include centrilobular hepatic necrosis in the liver, renal proximal tubular necrosis, and cardiac damage (Turgut et al., 2005). The poisonous component isolated and identified in the seeds of *X. strumarium* was carboxyatractyloside (CAT) (Cole et al., 1980; Turgut et al., 2005). Our literature search showed that the antioxidant activity of various extracts from various parts of *X. strumarium* has previously been reported. For example, chloroform, ethanol, and methanol extracts obtained from the roots of *X. strumarium* and 80% methanol, 85% methanol, 98% methanol, 80% ethanol, ethyl acetate, and chloroform/dichloromethane (1:1) extracts obtained from the leaves of *X. strumarium* have previously been reported for their DPPH radical scavenging activity (Guemmaz et al., 2018; Ishwarya & Singh, 2010; Rad et al., 2013; Scherer & Godoy, 2014; Sridharamurthy et al., 2011; Subba & Gaire, 2022). Other solvent extracts such as hexane, acetone, and water extracts of *X. strumarium* have not been investigated so far, especially the species collected in the Kingdom of Lesotho. Therefore, in the present study, we aimed to evaluate the antioxidant activity and ferric-reducing power of various solvent extracts obtained from the leaves and stem bark of *X. strumarium* collected in the Kingdom of Lesotho. Additionally, we also aimed to determine the total phenolic contents (TPCs) and total flavonoid contents (TFCs) of these solvent extracts obtained from the leaves and stem bark of *X. strumarium*. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing power assay were used to evaluate the antioxidant activity. In addition, the IC_{50} values of these solvent extracts were also determined by using DPPH radical scavenging assay. The TPCs and TFCs of these solvent extracts were determined by Folin-Ciocalteu colorimetric method and aluminum chloride colorimetric method, respectively, and the results are summarized in this article.

2. Materials and methods

2.1. Plant material

The plant material viz. fresh leaves and stem bark of *X. strumarium* were collected in January 2022 inside the Roma Campus of the National University of Lesotho (NUL), Lesotho, Southern Africa. Dr. Seleteng-Kose, Department of Biology, NUL, identified the plant material. A voucher specimen viz. Potlaki/XSLS/2022 and Potlaki/XSSB/2022 for leaves and stem bark, respectively, were kept separately at Organic Research Laboratory, Department of Chemistry & Chemical Technology, Faculty of Science & Technology (FoST), NUL, Roma Campus, Maseru, the Kingdom of Lesotho, Southern Africa.

2.2. Processing of plant material

The plant material was air-dried separately at room temperature for two weeks and then ground into powder using a blender (Waring Blender, Model HGB2WT93, 240V AC, 3.5 AMPs). Approximately, 1.850 and 1.800kg of powdered leaves and stem bark, respectively, were obtained.

2.3. Preparation of plant extracts

A mass of 250 g of powdered leaves was extracted with hexane for 24 hours at room temperature using a mechanical shaker. Using a vacuum filter (ATB, Model: 284065-H, Power: 230V 3.0A, 1320/min 50 Hz), the solution was filtered and the solvent was removed using a Buchi rotavapor. The hexane crude extract thus obtained was transferred to a pre-weighed beaker. The leaf powder was recovered from the above process and was extracted again with hexane at reflux conditions for 24 hours. A total mass of 4.576 g of combined hexane crude extract was obtained. The same procedure was followed separately to obtain crude extracts from other solvents. A mass of 8.098, 62.687, 6.262, 26.371, and 26.612 g chloroform, ethyl acetate, acetone, methanol, and water crude extracts was obtained, respectively, from 250, 250, 250, 250, and 95 g of powdered leaves. Similarly, 1.224, 0.865, 4.547, 2.242, 34.414, and 8.994 g of hexane, chloroform, ethyl acetate, acetone, methanol, and water stem-bark crude extracts were obtained, respectively, from 250, 250, 250, 250, 250 and 90 g of powdered stem-bark.

2.4. Chemicals used

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

2.5. DPPH radical scavenging activity and determination of 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 *X. strumarium* were achieved DPPH radical scavenging assay by using the method described in the literature (Pillai & Magama, 2020; Pillai et al., 2018; Sixtus & Pillai, 2022). 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) and further dilutions from each of these stock solutions (3000, 2000, 1500, 1000, 800, 500, and 200 µg/ml), the preparation of negative control (50% methanol blank solution, v/v) and rest of the experimental procedure were as per the details outlined in the literature (Sixtus & Pillai, 2022). Briefly, 3.94 mg of DPPH dissolved in 100 ml of methanol was served as an oxidant solution. The test solution consisted of 0.1 ml of each extract solution or positive control, 1.0 ml of 0.1 mM DPPH antioxidant solution, and 0.45 ml of 50 mM Tris-HCL buffer at pH 7.4. The absorbance of the mixture was measured at 517 nm after 30 min incubation. All experiments were conducted in triplicates and the results were reported as the average value of three experiments. The percentage of DPPH radical scavenging activity was calculated by the equation:

$$DPPH\ Scavenged\ (\%) = \frac{A_{Cont} - A_{Test}}{A_{Cont}} x\ 100$$

where A_{test} = Absorbance of extract solution or positive control, A_{cont} = Absorbance of negative control. The IC_{50} value of each extract and positive control were also determined as per the procedure outlined in the literature (Sixtus & Pillai, 2022), i.e. by plotting extract concentrations versus the percentage inhibition of DPPH radical,

which was obtained by averaging three experimental values (Sixtus & Pillai, 2022).

2.6. Ferric reducing power assay

The evaluation of the ferric-reducing power of various extracts obtained from the leaves and stem bark of *X. strumarium* was carried out using a method described in the literature (Sixtus & Pillai, 2022). 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), 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 (Sixtus & Pillai, 2022). Briefly, the reaction mixture consisted of 2.0 ml of each solution of extract or ascorbic acid, 2.0 ml of 0.2 M phosphate buffer at pH 6.6, and 2.0 ml of 0.01% potassium ferricyanide solution. A volume of 2.0 ml of 0.1% trichloroacetic acid was added to the reaction mixture after 20 minutes of incubation at 50 °C. The supernatant was collected separately after centrifugation of the mixture at 3000 rpm for 10 minutes. An aliquot of each supernatant 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 minutes. Ferric reducing power is the ability of an extract or pure compound to reduce Fe (III) to Fe (II) (Sixtus & Pillai, 2022). 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. The higher value of absorbance means a higher ferric reducing the power of an extract or pure compound and vice versa.

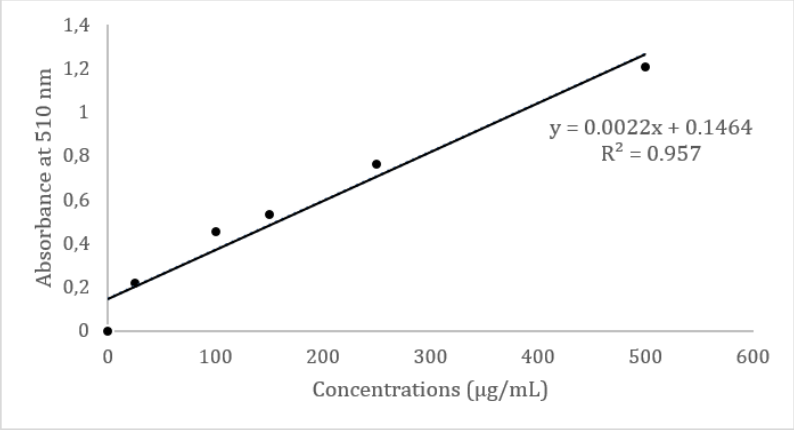

Figure 1. Calibration curve of gallic acid used to determine TPCs of various extracts

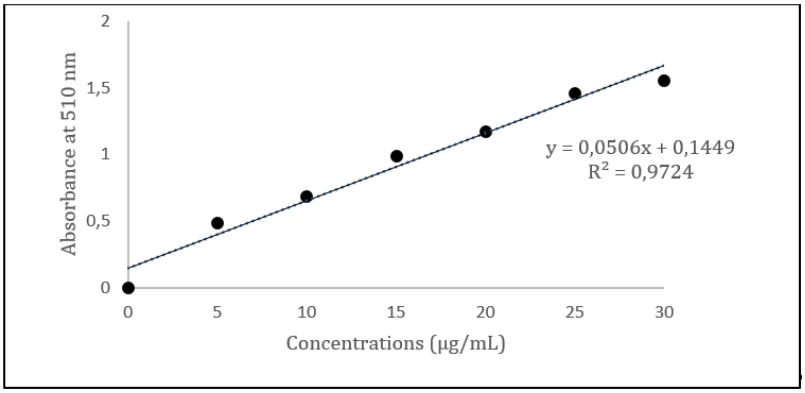

Figure 2. Calibration curve of quercetin used to determine TFCs of various extracts

2.7. Determination of total phenolic contents (TPCs)

Folin-Ciocalteu colorimetric method was employed to determine the TPCs of various extracts obtained from the leaves and stem-bark of *X. strumarium* as per literature (Sixtus & Pillai, 2022). Gallic acid served as a standard to get a calibration curve (Figure 1). The preparation of a test solution of each extract (10 ml at a concentration of 1000 µg/ml in 50% methanol, v/v), a stock solution of gallic acid (1000 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 (Sixtus & Pillai, 2022). 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 the 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 hours. The absorbance of each of these mixtures 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 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 + 0.1454; R^2 = 0.957) in a concentration range of 100-600 µg/ml is given in Figure 1.

2.8. Determination of total flavonoid contents (TFCs)

The aluminum chloride colorimetric method was employed to determine the TFCs of various extracts obtained from the leaves and stem bark of *X. strumarium* as per the literature (Sixtus & Pillai, 2022). Quercetin served as a standard to get a calibration curve (Figure 2). The preparation of a test solution of each extract (10 ml at a concentration of 1000 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 of the experimental procedure were as per the details outlined in the literature (Sixtus & Pillai, 2022). 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 the addition of 0.3 ml of 10% aluminum chloride after a 5 minutes 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 = 0.0506x + 0.1449; R^2 = 0.9724) in a concentration range of 5-30 µg/ml is given in Figure 2.

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) for ferric reducing power assay. The differences were statistically significant when $p \leq 0.05$.

3. Results and discussion

3.1. Evaluation of various extracts for their DPPH radical scavenging activity and determination of their IC_{50} values

The following twelve extracts were prepared from leaves and stem bark of *X. strumarium*. Hexane leaf extract (E1), chloroform leaf extract (E2), ethyl acetate leaf extract (E3), acetone leaf extract (E4), methanol leaf extract (E5), water leaf extract (E6), hexane stem bark extract (E7), chloroform stem bark extract (E8), ethyl acetate stem bark extract (E9), acetone stem bark extract (E10), methanol stem bark extract (E11) and water stem bark extract (E12). The result of the DPPH radical scavenging activity of all these twelve extracts (E1-E12) and the positive control, ascorbic acid are summarised in Tables 1 and 2. Ascorbic acid showed the highest radical scavenging activity of 88.98 ± 0.31% at a concentration of 3000 µg/ml (Tables 1 and 2). The leaf extracts showed the following order of scavenging activity: E1 > E2 > E5 > E4 > E3 > E6 (Tables 1 and 2). Extract, E1 showed the highest scavenging activity followed by E2 and E5, E4, E3, and E2 at a concentration of 3000 µg/ml (Tables 1 and 2). Similarly, the stem bark extracts exhibited the following order of scavenging activity: E8 > E10 > E7 > E9 > E11 > E12 (Tables 1 and 2). Extract, E8 showed the highest scavenging activity followed by E7, E10, E9, E11, and E12 at a concentration of 3000 µg/ml (Tables 1 and 2). Overall, all extracts (E1-E12) showed a moderate-to-significant radical scavenging activity albeit relatively lower than the positive control, ascorbic acid. Extracts, E1, E2, E5, and E8 showed > 80% scavenging activity at a concentration of 3000 µg/ml, which was comparable to positive control. The radical scavenging potential of these twelve extracts (E1-E12) and ascorbic acid are also shown in the bar diagrams for ease of comparison (refer to Figure 3 and Figure 4).

Additionally, the IC_{50} values of E1-E12 and ascorbic acid were determined and are summarised in Tables 1 and 2. The IC_{50} values of E1-E12 were determined to be < 200, < 200, 1856.02, 212.14, < 200, < 200, 2465.21, < 200, < 200, 1575.34, 256.74, 1939.04, and > 3000 µg/ml, respectively. The IC_{50} value of positive control, ascorbic acid, was determined to be < 200 µg/ml. This result showed that the leaf extracts viz. E1, E2, and E5 were found to be the most potent and their IC_{50} values were found to be < 200 for each extract, which was similar to positive control. Extracts, E4, E3, and E6 showed IC_{50} values of 212.14, 1856.02, and 2465.21 µg/ml, respectively. Similarly, the stem bark extracts viz. E7 and E8 were found to be the most potent and their IC_{50} value was found to be < 200 for each extract, which was similar to positive control. Extracts, E10, E9, E11, and E12 showed IC_{50} values of 212.14, 1856.02, 2465.21, and > 3000 µg/ml, respectively. Extract, E12 showed the lowest scavenging activity among all extracts with an IC_{50} value of > 3000 µg/ml. Overall, E1, E2, E5 E7, and E8 were identified as the most potent extracts with IC_{50} value of < 200 for each extract (Tables 1 and 2).

Various solvent extracts obtained from various parts of *X. strumarium* collected in different locations in several countries have previously been investigated for their DPPH free radical scavenging activity. For example, in a previous study, the dried roots of *X. strumarium* have been procured from a local market in the Karnataka State of India (Sridharamurthy et al., 2011). Chloroform and 90% ethanolic crude extracts have been obtained from these dried roots by cold maceration and Soxhlet apparatus methods, respectively (Sridharamurthy et al., 2011). The IC_{50} values of the chloroform and 90% ethanolic extracts have been determined to be 29.81 ± 0.95 and 24.85 ± 2.49 µg/ml, respectively in the DPPH radical scavenging assay (Sridharamurthy et al., 2011). Similarly, in another study, the roots of *X. strumarium* have been collected in the Tambaram area of Chennai, India (Ishwarya & Singh, 2010). Chloroform and methanolic crude extracts have been obtained from the dried roots by cold percolation and Soxhlet apparatus methods, respectively. The IC_{50} values of the chloroform and methanolic crude extracts have been determined to be 10.28 ± 0.69 and 40.40 ± 0.19 µg/ml, respectively in the DPPH radical scavenging assay (Ishwarya & Singh, 2010). Additionally, several fractions obtained from these chloroform and methanolic crude extracts have exhibited IC_{50} values in the ranges of 51.05 ± 3.61-180.40 ± 2.69 and 63.83 ± 0.23-184 ± 2.69 µg/ml, respectively in the DPPH radical scavenging assay (Ishwarya & Singh, 2010). In another study, the leaves of *X. strumarium* were collected in 2021 in the Arghakhanchi District of Nepal (Subba & Gaire, 2022). A methanolic crude extract has been obtained from the dried leaves by cold percolation method at room temperature and this methanolic extract exhibited an IC_{50} value of 258.10 µg/ml in the DPPH radical scavenging assay (Subba & Gaire, 2022). In another study, the leaves of *X. strumarium* were collected between August and September 2012 in the Baluchestan Province of Iran (Rad et al., 2013). An 85% methanolic extract has been obtained from the dried leaves by heating the content in a water bath at 25 °C for 24 hours with the help of a mechanical shaker (Rad et al., 2013). This 85% methanolic extract exhibited an IC_{50} value of 90 ± 0.01 µg/ml in the DPPH radical scavenging assay (Rad et al., 2013). In another study, the leaves of *X. strumarium* were collected in April 2014 on the farm of the Faculty of Agricultural Engineering, State University of Campinas, Campinas, Sao Paulo, Brazil (Scherer & Godoy, 2014). 80% ethanol, 80%

methanol, ethyl acetate, and chloroform/dichloromethane (1:1) extracts have been obtained from the air-dried leaves using static maceration, dynamic maceration and Soxhlet apparatus methods (Scherer & Godoy, 2014). The 80% ethanol extracts obtained from the static maceration, dynamic maceration, and Soxhlet apparatus methods were found to exhibit IC_{50} values of 47.83 ± 1.40, 53.01 ± 1.20 and 53.34 ± 1.52 µg/ml, respectively in the DPPH radical scavenging assay (Scherer & Godoy, 2014). However, the 80% methanol extracts obtained from the static maceration, dynamic maceration, and Soxhlet apparatus were found to demonstrate IC_{50} values of 44.94 ± 1.06, 45.05 ± 1.15 and 43.53 ± 1.61 µg/ml, respectively in the DPPH radical scavenging assay (Scherer & Godoy, 2014). Furthermore, the ethyl acetate extracts obtained from the static maceration, dynamic maceration, and Soxhlet apparatus methods showed IC_{50} values of 346.35 ± 16.50, 369.83 ± 13.58 and 423.97 ± 22.27 µg/ml, respectively in the DPPH radical scavenging assay (Scherer & Godoy, 2014). On the other hand, the chloroform/dichloromethane (1:1) extracts obtained from the static maceration and dynamic maceration presented IC_{50} values of 657.10 ± 24.01 and 674.61 ± 28.57 µg/ml, respectively (Scherer & Godoy, 2014). In another study, the leaves of *X. strumarium* were collected in July-August 2013 in Beni Aziz (Setif), Algeria (Guemmaz et al., 2018). A 98% methanolic crude extract has been obtained from the dried leaves by cold percolation method at room temperature and this 98% methanolic extract showed an IC_{50} value of 84.00 ± 0.0003 µg/ml in the DPPH radical scavenging assay. The chloroform, ethyl acetate, and aqueous fractions obtained from this 98% methanolic extract have demonstrated IC_{50} values of 234.00 ± 0.017, 17.00 ± 0.0004 and 46.00 ± 0.0006 µg/ml, respectively in the DPPH radical scavenging assay (Guemmaz et al., 2018).

Table 1. Percentage inhibition of DPPH radical scavenging activity of various extracts from leaves and stem-bark of *X. strumarium*

Extracts	Concentrations (µg/ml)/Inhibition (%)						
	200	500	800	1000	1500	2000	3000
E1	48.11 ± 3.86^{c}	53.04 ± 1.28^{e}	56.32 ± 2.18^{f}	62.15 ± 6.30^{f}	70.92 ± 2.99^{e}	76.48 ± 2.64^{d}	85.67 ± 11.54^{f}
E2	63.21 ± 0.09^{a}	65.57 ± 0.05^{b}	70.92 ± 0.09^{b}	74.94 ± 0.14^{a}	80.48 ± 0.14^{a}	82.26 ± 0.09^{a}	84.89 ± 0.27^{b}
E3	18.06 ± 0.31^{b}	26.77 ± 0.18^{a}	34.10 ± 1.03^{b}	43.76 ± 0.27^{b}	46.39 ± 0.34^{a}	55.59 ± 0.09^{a}	62.59 ± 0.09^{a}
E4	47.87 ± 0.09^{a}	52.60 ± 0.18^{b}	56.41 ± 0.14^{a}	58.75 ± 0.34^{a}	68.38 ± 0.34^{a}	74.05 ± 0.14^{a}	78.46 ± 0.18^{a}
E5	53.58 ± 0.14^{a}	60.37 ± 0.18^{a}	71.87 ± 0.18^{a}	73.94 ± 0.09^{a}	76.60 ± 0.09^{a}	80.28 ± 0.14^{a}	84.54 ± 0.27^{b}
E6	20.27 ± 0.22^{b}	23.20 ± 0.14^{a}	29.55 ± 0.27^{b}	36.79 ± 0.09^{a}	42.26 ± 0.14^{a}	48.08 ± 0.14^{a}	51.57 ± 0.18^{a}
E7	51.50 ± 1.01^{c}	57.39 ± 1.25^{d}	61.08 ± 0.35^{a}	62.80 ± 1.24^{c}	65.07 ± 0.41^{c}	66.05 ± 0.23^{b}	67.67 ± 2.09^{d}
E8	50.98 ± 0.46^{a}	56.29 ± 0.46^{b}	62.41 ± 0.31^{a}	71.07 ± 0.36^{a}	80.03 ± 0.10^{a}	82.99 ± 0.20^{b}	84.18 ± 0.92^{c}
E9	20.26 ± 0.44^{a}	35.93 ± 0.22^{a}	38.21 ± 0.23^{a}	46.01 ± 0.55^{a}	49.94 ± 0.44^{b}	63.03 ± 0.54^{c}	65.72 ± 0.18^{a}
E10	47.16 ± 0.49^{b}	47.99 ± 0.22^{a}	56.86 ± 0.14^{a}	62.23 ± 0.09^{b}	68.41 ± 0.14^{b}	73.73 ± 0.18^{a}	76.98 ± 0.59^{b}
E11	17.97 ± 0.41^{b}	27.22 ± 0.15^{a}	33.75 ± 0.18^{b}	41.78 ± 0.22^{d}	47.78 ± 0.09^{c}	54.26 ± 0.09^{a}	59.84 ± 0.09^{a}
E12	9.13 ± 0.54^{c}	12.12 ± 0.28^{c}	18.20 ± 0.14^{a}	21.69 ± 0.14^{a}	27.01 ± 0.18^{a}	35.52 ± 0.18^{b}	43.03 ± 0.18^{a}
Asc. acid	56.64 ± 1.26^{d}	65.69 ± 0.39^{a}	74.29 ± 0.09^{a}	77.33 ± 1.33^{d}	82.89 ± 0.09^{a}	84.78 ± 0.14^{a}	88.98 ± 0.31^{b}

E1 = hexane leaf extract, E2 = chloroform leaf extract, E3 = ethyl acetate leaf extract, E4 = acetone leaf extract, E5 = methanolic leaf extract, E6 = water leaf extract, E7= hexane stem bark extract, E8 = chloroform stem bark extract, E9 = ethyl acetate stem bark extract, E10 = acetone stem bark extract, E11 = methanolic stem bark extract, E12 = water stem bark extract, Asc. acid = Ascorbic acid, TFCs = Total flavonoid contents, TPCs = Total phenolic contents, N/A = Not applicable. Values with different superscript letters are statistically different within column.

Table 2. IC_{50} values, TPCs and TFCs of various extracts from leaves and stem-bark of *X. strumarium*

Extracts	IC_{50} (µg/ml)	TPCs (mg GAE/g DW)	TFCs (mg QE/g DW)
E1	< 200	4.03 ± 0.34	0.73 ± 0.03
E2	< 200	54.11 ± 1.16	5.04 ± 1.08
E3	1856.02	59.98 ± 2.01	3.01 ± 1.16
E4	212.14	118.01 ± 1.85	11.63 ± 2.32
E5	< 200	133.41 ± 3.23	20.61 ± 1.81
E6	2465.21	35.92 ± 5.00	1.20 ± 0.34
E7	< 200	6.89 ± 1.73	1.00 ± 0.78
E8	< 200	38.04 ± 0.88	3.76 ± 1.26
E9	1575.34	61.46 ± 1.11	3.91 ± 1.17
E10	256.74	60.80 ± 6.17	7.08 ± 2.35
E11	1939.04	121.21 ± 3.14	14.90 ± 1.18
E12	> 3000	29.42 ± 2.31	3.23 ± 0.79
Asc. acid	< 200	N/A	N/A

E1 = hexane leaf extract, E2 = chloroform leaf extract, E3 = ethyl acetate leaf extract, E4 = acetone leaf extract, E5 = methanolic leaf extract, E6 = water leaf extract, E7= hexane stem bark extract, E8 = chloroform stem bark extract, E9 = ethyl acetate stem bark extract, E10 = acetone stem bark extract, E11 = methanolic stem bark extract, E12 = water stem bark extract, Asc. acid = Ascorbic acid, TFCs = Total flavonoid contents, TPCs = Total phenolic contents, N/A = Not applicable. Values with different superscript letters are statistically different within column.

From the above discussions, we noticed that cold maceration or cold percolation, or hot solvent extraction techniques have been utilized to obtain various solvent extracts from various parts of *X. strumarium* collected at different locations in several countries and these solvent extracts have been evaluated for their antioxidant activity by DPPH radical scavenging assay (Guemmaz et al., 2018; Ishwarya & Singh, 2010; Rad et al., 2013; Scherer & Godoy, 2014; Sridharamurthy et al., 2011; Subba & Gaire, 2022). However, in the present study, the combination of maceration and hot solvent extraction techniques was used to obtain various solvent extracts from the leaves and stem bark of *X. strumarium* collected in the Kingdom of Lesotho. The maceration technique was first used to extract compounds as much as possible from the plant materials and the sample recovered from the maceration technique was further subjected to hot solvent extraction at reflux conditions to extract the remaining compounds. In other words, the optimal extraction of compounds from the plant materials was achieved by the combination of these two extraction techniques. Additionally, these solvent extracts from the leaves and stem bark of *X. strumarium* obtained from the above extraction techniques were evaluated for their DPPH radical scavenging activity. We noticed that the methanol and ethyl acetate extracts obtained from the leaves of *X. strumarium* were common both in the previous and present studies (Scherer & Godoy, 2014; Subba & Gaire, 2022). As

discussed previously that the methanolic extract obtained from the leaves of *X. strumarium* collected in 2021 in Nepal by cold percolation method was found to exhibit an IC_{50} value of 258.10 µg/ml in the DPPH radical scavenging assay (Subba & Gaire, 2022). Similarly, the ethyl acetate extract obtained from the leaves of X. *strumarium* collected in April 2014 in Brazil by static maceration, dynamic maceration, and Soxhlet apparatus methods showed IC_{50} values of 346.35 ± 16.50, 369.83 ± 13.58 and 423.97 ± 22.27 µg/ml, respectively in the DPPH radical scavenging assay (Scherer & Godoy, 2014). However, in the present study, the methanol and ethyl acetate extracts obtained from the leaves of *X. strumarium* collected in January 2022 in the Kingdom of Lesotho exhibited IC_{50} values of < 200 and 1856.02 µg/ml, respectively in the same DPPH radical scavenging assay. In other words, in the present study, the methanolic extract from the leaves of *X. strumarium* demonstrated significantly higher radical scavenging activity compared to the previous report (Subba & Gaire, 2022) and therefore, it displayed a lower IC_{50} value. On the other hand, in the present study, the ethyl acetate extract obtained from the leaves of *X. strumarium* possessed much lower scavenging activity compared to the previous report (Scherer & Godoy, 2014) and therefore, it exhibited a much higher IC_{50} value. This observation revealed a fact that there was a discrepancy in the IC_{50} values of methanol and ethyl acetate extracts reported in the previous studies (Scherer & Godoy, 2014; Subba & Gaire, 2022) and the determined IC_{50} values in the present study. This discrepancy can be attributed to the facts that the quantity, variety, and variation of active compounds that could be extracted from the plant materials are dependent on the geographic locations and seasons in which the plant materials are collected as well as the extraction techniques used to obtain various extracts from the collected plant materials. The discrepancy in the IC_{50} values of methanol and ethyl acetate extracts in the previous (Scherer & Godoy, 2014; Subba & Gaire, 2022) and present studies might be due to these factors.

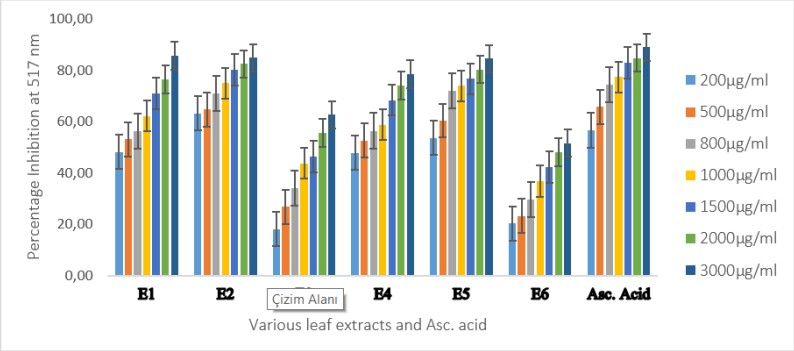

Figure 3. Percentage of DPPH radical scavenging activity of various extracts from leaves of *X. strumarium* and ascorbic acid at various concentrations

E1 = hexane leaf extract, E2 = chloroform leaf extract, E3 = ethyl acetate leaf extract, E4 = acetone leaf extract, E5 = methanolic leaf extract, E6 = water leaf extract, Asc. acid = Ascorbic acid

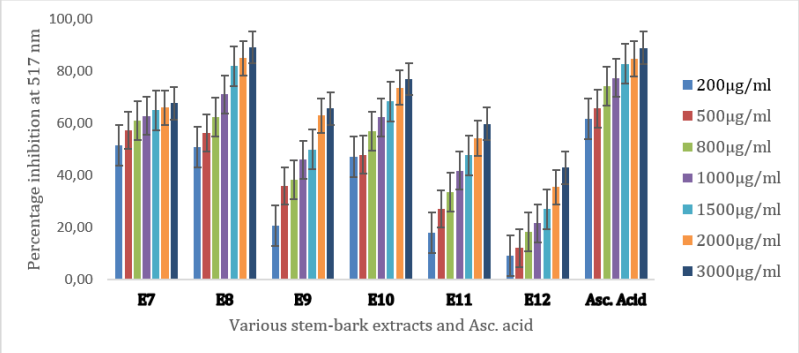

Figure 4. Percentage of DPPH radical scavenging activity of various extracts from stem-bark of *X. strumarium* and ascorbic acid at various concentrations

E7 = hexane stem-bark extract, E8 = chloroform stem-bark extract, E9 = ethyl acetate stem-bark extract, E10 = acetone stem-bark extract, E11 = methanolic stem-bark extract, E12 = water stem-bark extract. Asc. acid = Ascorbic acid

3.2. Evaluation of various extracts for their ferric-reducing power

The result of the ferric-reducing power of all twelve extracts (E1-E12) is summarised in Table 3. Ascorbic acid served as the positive control. For clarity and comparison purposes, the dose-response curve of ferric-reducing power of various leaves extracts (E1-E6) and stem bark extracts (E7-E12) are given in Figures 5 and 6, respectively together with the dose-response curve for positive control, ascorbic acid. In general, the stem bark extracts (E7-E12) showed higher ferric-reducing power than leaf extracts (E1-E6). The ferric-reducing power of the leaf extracts was observed in the following order: E3 > E5 > E4 > E1 > E6 > E2. Extract, E3 exhibited the highest reducing power of 0.996 ± 0.101 at a concentration of 100 µg/ml. Similarly, the ferric-reducing power of the stem bark extracts was observed in the following order: E9 > E10 > E11 > E12 > E7 > E8. Extract E9 showed the highest ferric-reducing power of 0.947 ± 0.018 at a concentration of 100 µg/ml. Extracts E10 and E11 also showed comparable ferric-reducing power as that of E9. However, all extracts (E1-12) showed lower ferric-reducing power than the positive control, ascorbic acid at higher concentrations. In a

previous report, a methanolic crude extract and its chloroform, ethyl acetate, and aqueous fractions obtained from the leaves of *X. strumarium* have exhibited EC_{50} values of 0.059 ± 0.001, 0.120 ± 0.0045, 0.017 ± 0.00015 and 0.036 ± 0.0016 mg/ml, respectively in the ferric-reducing power assay (Guemmaz et al., 2018).

Table 3. Ferric reducing power of various extracts from leaves and stem bark of *X. strumarium**

Extracts	Concentrations (µg/ml)/Ferric reducing power					
	5	10	20	40	80	100
E1	0.125 ± 0.034^{c}	0.186 ± 0.003^{a}	0.280 ± 0.012^{a}	0.384 ± 0.065^{e}	0.502 ± 0.027^{c}	0.611 ± 0.073^{e}
E2	0.083 ± 0.010^{a}	0.114 ± 0.018^{b}	0.178 ± 0.002^{a}	0.290 ± 0.041^{d}	0.312 ± 0.007^{a}	0.394 ± 0.045^{d}
E3	0.361 ± 0.007^{a}	0.397 ± 0.004^{a}	0.455 ± 0.044^{c}	0.691 ± 0.023^{b}	0.884 ± 0.078^{f}	0.996 ± 0.101^{f}
E4	0.224 ± 0.052^{d}	0.296 ± 0.043^{d}	0.383 ± 0.008^{a}	0.472 ± 0.038^{b}	0.567 ± 0.057^{e}	0.709 ± 0.064^{e}
E5	0.302 ± 0.046^{d}	0.366 ± 0.001^{a}	0.444 ± 0.074^{e}	0.678 ± 0.029^{c}	0.753 ± 0.035^{c}	0.922 ± 0.005^{a}
E6	0.111 ± 0.012^{a}	0.143 ± 0.093f	0.194 ± 0.003^{a}	0.298 ± 0.000^{a}	0.346 ± 0.041^{d}	0.412 ± 0.009^{a}
E7	0.138 ± 0.037^{c}	0.176 ± 0.082^{f}	0.266 ± 0.015^{a}	0.352 ± 0.013^{a}	0.498 ± 0.032^{c}	0.593 ± 0.001^{a}
E8	0.093 ± 0.004^{a}	0.125 ± 0.000^{a}	0.155 ± 0.012^{a}	0.226 ± 0.019^{b}	0.302 ± 0.019^{b}	0.361 ± 0.024^{b}
E9	0.241 ± 0.034^{a}	0.317 ± 0.008^{a}	0.478 ± 0.053^{e}	0.513 ± 0.002^{a}	0.728 ± 0.034^{c}	0.947 ± 0.018^{b}
E10	0.192 ± 0.006^{a}	0.290 ± 0.012^{a}	0.368 ± 0.042^{d}	0.552 ± 0.001^{a}	0.800 ± 0.013^{a}	0.911 ± 0.089f
E11	0.202 ± 0.063^{e}	0.287 ± 0.004^{a}	0.406 ± 0.013^{a}	0.623 ± 0.030	0.764 ± 0.011^{a}	0.885 ± 0.004^{a}
E12	0.103 ± 0.016^{b}	0.152 ± 0.007^{a}	0.238 ± 0.007^{a}	0.426 ± 0.017^{b}	0.576 ± 0.014a	0.660 ± 0.034^{c}
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^{e}

* Refer to footnotes of Tables 1 and 2

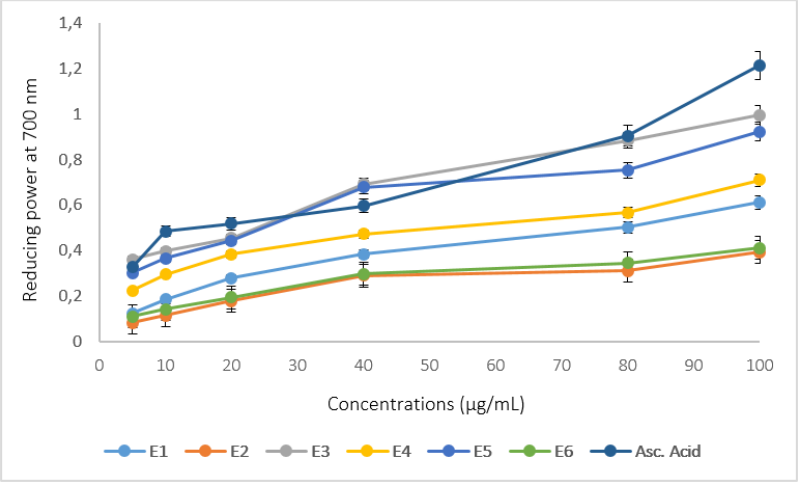

Figure 5. Dose-response curve for various extracts (E1-E6) obtained from leaves of *X. strumarium* for their ferric reducing power
Refer the footnotes of Figure 3

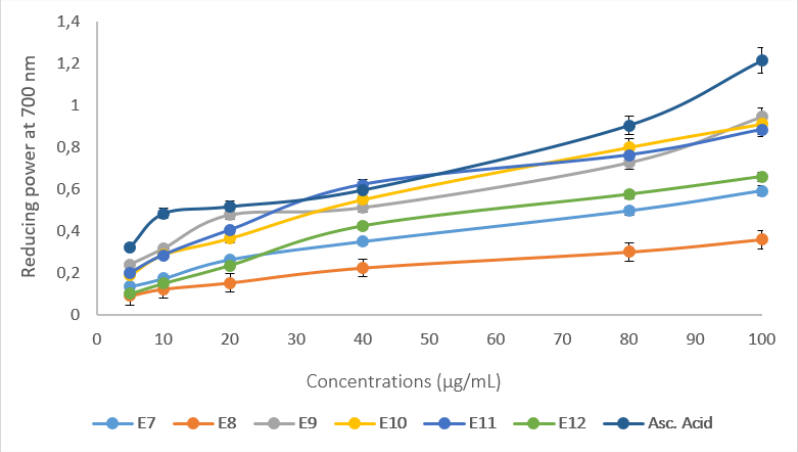

Figure 6. Dose-response curve for various extracts (E7-E12) obtained from stem-bark of *X. strumarium* for their ferric reducing power
Refer the footnotes of Figure 4

3.3. Determination of TPCs and TFCs of various extracts

The TPCs of E1-E12 were determined by Folin-Ciocalteu colorimetric method and the results are summarized in Tables 1 and 2. Gallic acid served as a standard to estimate the TPCs of various extracts and the calibration curve of gallic acid is given in Figure 1. The TPCs of extracts from leaves (E1-E6) were found to be 4.03 ± 0.34, 54.11 ± 1.16, 59.98 ± 2.01, 118.01 ± 1.85, 133.41 ± 3.23 and 35.92 ± 5.00 mg of GAE/g DW, respectively. This result showed that E5 exhibited the highest TPCs among leaf extracts followed by E4, E3, E2, E6, and

E1 (Table 3). The TPCs of extracts from stem bark (E7-E12) were found to be 6.89 ± 1.73, 38.04 ± 0.88, 61.46 ± 1.11, 60.80 ± 6.17, 121.21 ± 3.14 and 29.42 ± 2.31 mg of GAE/g DW, respectively. This result indicated that E11 showed the highest TPCs among stem bark extracts followed by E9, E10, E8, E12, and E7 (Tables 1 and 2).

The TFCs of E1-E12 were determined by the aluminum chloride colorimetric method and the results are summarized in Tables 1 and 2. Quercetin served as a standard to estimate the TFCs of various extracts and the calibration curve of quercetin is given in Figure 1. The TFCs of extracts from leaves (E1-E6) were found to be 4.03 ± 0.34, 54.11 ± 1.16, 59.98 ± 2.01, 118.01 ± 1.85, 133.41 ± 3.23 and 35.92 ± 5.00 mg of QE/g DW, respectively. This result showed that E5 exhibited the highest TFCs among leaf extracts followed by E4, E3, E2, E6, and E1 (Tables 1 and 2). The TFCs of extracts from stem bark (E7-E12) were found to be 6.89 ± 1.73, 38.04 ± 0.88, 61.46 ± 1.11, 60.80 ± 6.17, 121.21 ± 3.14 and 29.42 ± 2.31 mg of QE/g DW, respectively. This result indicated that E11 showed the highest TPCs among stem bark extracts followed by E10, E9, E8, E17, and E7 (Tables 1 and 2).

The TPCs and TFCs of various extracts obtained from various parts of the *X. strumarium* have previously been reported. For example, in a previous study, as discussed previously that the aerial parts of *X. strumarium* have been collected in May 2018 in Ho Chi Minh City, Vietnam (Ly et al., 2021). An ethanolic extract has been obtained from these aerial parts by cold percolation technique at room temperature. The TPCs and TFCs of this ethanolic extract have been determined to be 84.86 ± 5.13 mg of GAE/g DW and 3.66 ± 0.08 mg of QE/g DW, respectively (Ly et al., 2021). Similarly, in another study, as discussed previously that the leaves of *X. strumarium* were collected in July-August 2013 in the Beni Aziz (Setif), Algeria (Guemmaz et al., 2018). A 98% methanolic crude extract has been obtained from the dried leaves by cold percolation method at room temperature and the chloroform, ethyl acetate, and aqueous fractions have been obtained from this 98% methanolic crude extract (Guemmaz et al., 2018). The 98% methanolic crude extract, the chloroform, ethyl acetate, and aqueous fractions demonstrated TPCs of 85.77 ± 4.98, 0.58 ± 3.3, 166.26 ± 27.98 and 75.24 ± 13.31 mg of GAE/g DW, respectively (Guemmaz et al., 2018). Similarly, the 98% methanolic crude extract, the chloroform, ethyl acetate, and aqueous fractions displayed TFCs 11.76 ± 1.39, 17.26 ± 2.75, 29.037 ± 3.14 and 10.60 ± 1.615 mg of QE/g DW, respectively (Guemmaz et al., 2018). In another study, the leaves of *X. strumarium* were collected in April 2014 in Sao Paulo, Brazil (Scherer & Godoy, 2014). 80% ethanol, 80% methanol, ethyl acetate, and chloroform/dichloromethane (1:1) extracts have been obtained from the air-dried leaves using static maceration, dynamic maceration and Soxhlet apparatus methods (Scherer & Godoy, 2014). The TPCs of 80% ethanol extract obtained from the static maceration, dynamic maceration, and Soxhlet apparatus methods have been determined to be 64.51 ± 1.0, 70.07 ± 1.6 and 69.38 ± 1.3 mg GAE/g DW, respectively (Scherer & Godoy, 2014). Similarly, the TPCs of 80% methanol extract obtained from the static maceration, dynamic maceration, and Soxhlet apparatus methods have been determined to be 93.68 ± 2.1, 78.23 ± 0.9 and 81.35 ± 1.3 mg GAE/g DW, respectively (Scherer & Godoy, 2014). Additionally, the TPCs of ethyl acetate extract obtained from the static maceration, dynamic maceration, and Soxhlet apparatus methods have been determined to be 21.98 ± 3.6, 27.19 ± 1.0 and 23.19 ± 0.3 mg GAE/g DW, respectively (Scherer & Godoy, 2014). Furthermore, the TPCs of chloroform/dichloromethane (1:1) extract obtained from the static maceration and dynamic maceration have been determined to be 13.30 ± 0.8 and 18.85 ± 2.4 mg GAE/g DW, respectively (Scherer & Godoy, 2014). In the present study, we determined the presence of significant amounts of TPCs and TFCs in various extracts obtained from the leaves and stem bark of *X. stramarium*, and particularly, the methanolic extracts from both leaves and stem bark showed higher TPCs and TFCs. Moreover, we noticed that the ethyl acetate extracts obtained from the leaves of *X. strumarium* were common both in the previous and present studies. As discussed previously that the TPCs of ethyl acetate extract obtained from the leaves of *X. strumarium* collected in Brazil by static maceration, dynamic maceration, and Soxhlet apparatus methods have been determined to be 21.98 ± 3.6, 27.19 ± 1.0 and 23.19 ± 0.3 mg GAE/g DW, respectively. On the other hand, in the present study, TPCs of ethyl acetate extract obtained from the leaves of *X. strumarium* collected in the Kingdom of Lesotho were determined to be 59.98 ± 2.01 mg of GAE/g DW. In other words, the TPCs of ethyl acetate extract in the present study were found to be much higher than in the previous report. Again, this discrepancy might be due to the same factors that the quantity, variety, and variation of active compounds that could be extracted from the plant materials are dependent on the geographic locations and seasons in which the plant materials are collected as well as the extraction techniques used to obtain various extracts from the collected plant materials.

4. Conclusions

Various solvent extracts obtained from the leaves and stem bark of *X. strumarium* were evaluated for their DPPH radical scavenging activity and ferric-reducing power. The extracts from the leaves and stem bark exhibited radical scavenging activity in the ranges of 18.06 ± 0.3-185.67 ± 11.54% and 9.13 ± 0.54-84.18 ± 0.92%, respectively at a concentration range of 200-3000 µg/ml. The positive control, ascorbic acid exhibited radical scavenging activity in a range of 56.64 ± 1.26-88.98 ± 0.31% at the same concentration range of 200-3000 µg/ml. The hexane, chloroform, and methanol extracts from leaves and the chloroform extract from stem bark showed higher radical scavenging activity compared to other extracts at a concentration of > 3000 µg/ml. Additionally, the IC_{50} values of all these extracts were determined using the same DPPH radical scavenging assay. The hexane and chloroform extracts from both leaves and stem bark and the methanol extract from leaves were found to be the most potent extracts with an IC_{50} value of < 200 µg/ml for each extract. The positive control, ascorbic acid also showed an IC_{50} value of < 200 µg/ml. Furthermore, in the ferric-reducing power assay, the ethyl acetate extract from both leaves and stem bark exhibited the highest reducing power of 0.996 ± 0.101 and 0.947 ± 0.018 at a concentration of 100 µg/ml. Additionally, the methanol extract from the leaves showed the highest TPCs of 133.41 ± 3.23 mg GAE/g of DW of extract followed by the methanol extract from the stem bark and then the acetone extract from the leaves with TPCs of 121.21 ± 3.14 and 118.01 ± 1.85 mg GAE/g of DW of extract, respectively. Similarly, the methanol extracts from both leaves and stem bark also showed the highest TFCs of 20.61 ± 1.81 and 14.90 ± 1.18mg QE/g of DW of extract, respectively. Based on our findings, it can be concluded that diverse extracts derived from the leaves and stem bark of *X. strumarium* displayed a moderate-to-strong radical scavenging activity and ferric-reducing power, along with substantial levels of total phenolic compounds (TPCs) and total flavonoid contents (TFCs). Since *X. stramarium* has been used in traditional medicine to treat a variety of diseases, further studies on this plant are recommended to commercialize products from this plant.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Manoharan Karuppiah Pillai: Conceptualization, Supervision, Investigation, Data analysis, Compilation and writing the original draft
Potlaki Thabe: Conceptualization, Investigation, Data analysis and writing a draft
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Supplementary File

None.

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