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2,2-Diphenyl-1-picrylhydrazil radical scavenging activity of extracts from roots and leaves of *Searsia burchellii*

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Abstract

Searsia burchellii finds therapeutic applications in traditional medicine. Methanolic extracts, hexane, chloroform, ethyl acetate and methanol/water fractions of methanolic extracts and water extracts were obtained separately from the roots and leaves of *Searsia burchellii* by the combination of maceration, hot solvent extraction and solvent-solvent partition techniques. These extracts were evaluated for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazil radical scavenging assay (DPPH). The extracts from roots and their fractions showed radical scavenging activity ranging from 6.60±4.50 to 63.27±1.93% at various concentrations. Similarly, the extracts from leaves and their fractions showed radical scavenging activity ranging from 3.32±0.95 to 64.91±0.15% at various concentrations. Ascorbic acid served as positive control which showed radical scavenging activity ranging from 53.62±2.80 to 60.82±0.62% at various concentrations. The IC_{50} values of these extracts and fractions were found to be < 200 to > 3000 µg/mL. The IC_{50} value of ascorbic acid was found to be <200 µg/mL. From this study, we concluded that extracts and their fractions from *S. burchellii* showed promising radical scavenging activity.

1. Introduction

Searsia burchellii belongs to the Anacardiaceae family of the Searsia (*Rhus*) genus. *S. burchellii* is widely distributed in South Africa, Lesotho and Namibia (Palmer and Pitman, 1972; Moffett, 1994; Palgrave, 2002). The vernacular names of this plant are Karoo kuni -bush, Karookoeniebos, Motshotlho and Mokhoamphiri (Smith, 1966; Van Wyk and Gericke, 2000; Germishuizen and Meyer, 2003; Moffet, 2007). *S. burchellii* is an evergreen shrub and grows up to 5 meters in height. *S. burchellii* has densely populated olive green waxy leaves, grey coloured stem-bark, cream-colored flowers of 4-6 cm long and yields reddish-brown fleshy fruits (Compton, 1976; Van Wyk and Van Wyk, 1997; Van Oudtshroorn and Gericke, 1997; New Winger, 2000; Palgrave, 2002). *S. burchellii* has been used to treat tuberculosis, respiratory and pulmonary diseases, fever, cold and problems associated with childbirth (Nielsen *et al*., 2012; Umberto, 2012). Plants from the Searsia genus have been shown to possess anti-inflammatory, antimalarial, antimicrobial, antidiarrheal, anticancer, antiviral, hepatoprotective and antioxidant activities (Djakpo and Yao, 2010; Moteetee and Van Wyk, 2011; El-Salam and Mohammed, 2015; Moteetee and Kose, 2017; Mtunzi *et al*., 2017). However, the species *S. burchellii*, which belongs to the same Searsia genus, has not been explored for its biological and pharmacological activities so far. The aim of the current study was to evaluate the 2,2-diphenyl-1-picrylhydrazil radical scavenging activity of various organic solvents and aqueous extracts from roots and leaves of *S. burchellii* collected from the kingdom of Lesotho and to determine their IC_{50} values.

2. Materials and methods

2.1 Plant materials

The roots and leaves of *S. burchellii* were collected in January and March 2020 along the Senqu River at Ha Ramatseliso village in Thaba-Tseka district and on the hills of Maseru district in the Kingdom of Lesotho, Southern Africa. The plant materials were identified by Dr Lerato Seleteng-Kose, Department of Biology, National University of Lesotho, Roma campus, The Kingdom of Lesotho.

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2.2 Processing of plant materials

The plant materials were air-dried at room temperature for three weeks at Organic Chemistry Laboratory and then pulverized into powder using a grinding machine (HSIANGTAI, Mode SM-450L, AC220V, 50HZ, MRC Laboratory Equipment). A mass of 1160.83 and 997.17 g of powdered roots and leaves, respectively were obtained.

2.3 Preparation of plant extracts and fractions

A mass of 854.31 g of powdered leaves was macerated with 3.5 L of methanol for 72 hrs. The resulting solution was filtered off through a Whatman no.1 filter paper and a methanolic crude leaf extract was obtained. The above procedure was repeated twice and the extract was combined. Finally, the same material was extracted under reflux condition (about 65°C) for 10 hrs and the solution was concentrated. A mass of 244.33 g of combined methanolic crude leaf extract was obtained. Water extract from the leaves was obtained by boiling 142.86 g of powder with 300 mL of distilled water at 80° C for 10 hrs. A mass of 20.23 g of water leaf extract was obtained on concentration. Using a similar extraction procedure, a mass of 84.05 and 7.29 g of methanolic and water extracts, respectively were obtained from 1075.37 and 85.46 g of root powder. A mass of 5.15 and 85.15 of these methanolic crude extracts from roots and leaves, respectively were kept separately for their radical scavenging activity and other studies.

A mass of 159.179 g crude methanolic leaf extract was suspended in methanol-water (1:1 v/v) and subjected to solvent-solvent partition successively with hexane, chloroform and ethyl acetate. A mass of 18.953, 102.283 and 9.410 g of hexane, chloroform and ethyl acetate fractions, respectively were obtained. A mass of 22.879 g of the remaining methanol-water fraction was also obtained on concentration. Similarly, 2.703, 5.164, 15.856 and 46.548 g of hexane, chloroform, ethyl acetate and methanol-water fraction, respectively were obtained from 78.899 g crude methanolic root extract.

2.4 Chemicals used

AR Grade of hexane, chloroform, ethyl acetate, methanol, tris(hydroxymethyl)aminomethane and 2,2-diphenyl-1-picrylhydrazil (DPPH) were all purchased from Sigma-Aldrich. AR Grade of L-ascorbic acid was purchased from Minema Ltd.

2.5 Evaluation of DPPH radical scavenging activity of various extracts and their fractions and determination of their IC_{50} values

DPPH radical scavenging activity of various extracts and fractions from the leaves and roots of *S. burchellii* was determined by methods described in the literature (Mokoroane *et al*., 2020; Matamane *et al*., 2020) with slight modifications. Briefly, stock solutions of crude extracts and fractions were prepared separately by dissolving 30 mg in 10 mL 50% methanol (v/v). Further dilutions such as 3000, 2000, 1500, 1000, 800, 500 and 200 µg/mL were prepared from the stock solutions. The solutions without extracts or fractions served as negative controls. A volume of 0.1 mL of each extract solution was mixed separately with 1.0 mL of 0.1 mM DPPH solution and 1.0 mL of 50mM Tris-HCL buffer (pH 7.40) solution. The resulting mixture was vortexed and then incubated at room temperature in the dark for 30 min. A stock solution of ascorbic acid (0.3 g) in 50% methanol (v/v) was prepared and serial dilutions were made as previously which served as a positive control. The absorbance of the mixture was measured at 517 nm using MRM Spectrophotometer (Mode Spectro UV-11, S/N: UEB 1704200). The assays were performed in triplicates. The percentage inhibition activity of extracts or fractions was calculated by the formula: $[(A_{cont} - A_{test})/A_{cont}) \times 100]$, where A_{cont} is the absorbance of the negative control and A_{test} is the absorbance of the extract or fraction or positive control.

The IC_{50} values of these extracts or fractions were calculated using Microsoft Excel by plotting extract concentration versus percentage inhibition of DPPH radical. Each experiment was carried out in triplicate and the average of the three values was used to calculate the IC_{50} value for each extract. The IC_{50} value is defined as the concentration of extract that inhibits the formation of DPPH radical by 50% (Moyo *et al*., 2013; Ndhlala *et al*., 2013). A lower value of IC_{50} represents higher antioxidant activity and vice versa. Standard deviation was calculated for each concentration from the three values of the experiments.

2.6 Statistical analysis

Data analysis was performed using the SPSS 17.0 statistics program by means of a two-way analysis of variance. The differences were considered statistically significant when $p \leq 0.05$.

3. Results and discussion

The methanolic crude extract (E1) was prepared from the roots of *S. burchellii* and four fractions were obtained from this methanolic crude extract *viz*. hexane fraction (E2), chloroform fraction (E3), ethyl acetate fraction (E4) and methanol-water (E5). Additionally, water extract (E6) was also prepared separately from the root powder. Similarly, methanolic crude extract E7) was

prepared from the leaves of *S. burchellii* and four fractions were obtained from this methanolic crude extract viz. hexane fraction (E8), chloroform fraction (E9), ethyl acetate fraction (E10) and methanol-water fraction (E11). Additionally, water extract (E12) was also prepared separately from the powdered leaves. All these extracts and fractions were evaluated for their antioxidant activity at various concentrations by DPPH radical scavenging assay and the results are summarised in Table 1. Ascorbic acid served as a positive control.

Extract E1 showed slightly lower scavenging activity than positive control at all concentrations. Fraction E2 exhibited much lower radical scavenging activity at lower concentrations and showed significant radical scavenging activity at higher concentrations at 2000 and 3000 µg/mL. Fraction E3 showed lower radical scavenging activity at a lower concentration at 200 µg/mL and showed higher radical scavenging activity at higher concentrations at 500-3000 µg/mL. Fractions E4 and E5 exhibited higher radical scavenging activity as compared to the positive control at all concentrations. Extract E6 showed comparable radical scavenging activity as that of positive control at all concentrations. Extract 7 showed lower radical scavenging activity than positive control at all concentrations. Fractions E8 and E9 exhibited much lower radical scavenging activity at all concentrations. Fraction E10 showed a lower radical scavenging activity at 200 µg/mL and showed higher radical scavenging activity at 500-3000 µg/mL compared to the positive control. Fraction E11 showed a lower radical scavenging activity at lower concentrations at 200-500 µg/mL and showed higher radical scavenging activity at higher concentrations at 800-3000 µg/mL. Extract E12 showed lower radical scavenging activity at all concentrations relative to the positive control (Table 1). The DPPH radical scavenging activity of various extracts from roots and leaves of *S.burchellii* were evaluated. Among the root extracts, E4 and E5 showed remarkable radical scavenging activity. Among the leaf extracts, E10 and E11 showed promising radical scavenging activity. This is the first report of this kind, particularly the species collected from the Kingdom of Lesotho.

Additionally, the IC_{50} values of these extracts and fractions were also determined and the results are presented in Table 2. Extracts and fractions, E1-E12 showed IC_{50} values <200, 2469.87, 344.86, <200, <200, 623.33, 2082.63, >3000, >3000, <200, 292.80 and >3000 µg/mL, respectively (Table 2). The positive control, ascorbic acid showed an IC_{50} value of <200 µg/mL (Table 2). Extract E1 and fractions E4, E5 and E10 showed IC_{50} value of <200 µg/mL. Fractions E4 and E5 showed higher radical scavenging activity at all concentrations compared to positive control. The solvents *viz*. methanol, ethyl acetate and methanol/water might have higher extracting power of active ingrdients. These extracts or frations might have more active ingredients even at lower concentrations and therefore showed higher radical scavenging activity. They also showed a gradual increase in radical scavenging activity with increasing concentrations.

As stated previously that *S. burchellii* has not been studied previously. However, the antioxidant activity of other species from the *Searsia* genus has previously been reported. For example, various extracts from leaves and stem-bark of *Searsia leptodictya* and *Searsia tripartita* have been reported to have promising radical scavenging

Table 1. The percentage inhibition of DPPH radical scavenging activity of various extracts and fractions from roots and leaves of *S. burchellii*

Extracts	Concentrations (µg/mL)/ % inhibition						
	200	500	800	1000	1500	2000	3000
E1	49.40±4.54^{b}	54.98±4.47^{c}	55.86±3.92^{d}	56.48±4.17^{b}	57.13±3.81^{e}	57.68±3.35^{e}	58.00±3.31^{e}
E2	6.60±4.50^{e}	18.93±2.14^{c}	27.23±1.64^{a}	33.53±4.52^{c}	43.51±1.61^{d}	47.89±2.07^{c}	50.86±1.56^{c}
E3	30.19±2.53^{c}	56.69±2.26^{b}	61.70±1.70^{c}	62.00±1.57^{c}	62.31±1.16^{a}	62.70±1.26^{c}	63.27±1.93^{c}
E4	57.56±4.23^{a}	59.45±3.27^{a}	60.32±2.45^{c}	61.24±2.47^{c}	61.89±2.28^{b}	62.45±2.24^{c}	63.11±1.77^{b}
E5	55.00±0.60^{b}	57.99±1.05^{a}	59.55±0.44^{a}	60.32±1.03^{a}	61.40±0.84^{b}	62.56±0.58^{a}	63.14±0.84^{a}
E6	45.43±2.64^{a}	49.22±2.06^{a}	51.74±2.28^{a}	53.41±2.50^{c}	54.35±2.92^{a}	55.52±1.96^{b}	56.59±1.48^{a}
E7	9.74±3.96^{c}	22.60±3.41^{d}	25.24±3.88^{c}	31.68±1.96^{a}	46.73±1.42^{c}	55.61±2.69^{c}	58.48±0.90^{b}
E8	6.00±1.92^{b}	6.89±2.01^{e}	7.91±2.49^{c}	8.65±2.69^{b}	9.86±1.53^{c}	10.44±2.09^{e}	11.63±2.90^{b}
E9	3.32±0.95^{a}	6.45±1.50^{c}	8.09±3.35^{e}	11.25±4.01^{b}	18.05±2.06^{c}	22.69±0.65^{b}	25.92±2.96^{b}
E10	34.17±4.10^{e}	59.24±0.68^{a}	61.61±0.18^{b}	63.39±0.35^{a}	63.98±0.36^{b}	64.49±0.12^{a}	64.91±0.15^{c}
E11	40.34±6.41^{b}	50.87±6.54^{d}	56.93±4.66^{b}	60.97±0.82^{a}	63.69±1.20^{a}	64.37±1.26^{b}	64.88±1.48^{a}
E12	17.27±6.29^{c}	25.00±4.16^{b}	27.42±2.20^{a}	28.72±2.43^{b}	29.30±2.35^{b}	30.27±2.74^{c}	32.32±2.90^{b}
Ascorbic acid	53.62±2.80^{a}	54.91±2.55^{a}	56.43±2.19^{a}	57.44±1.56^{c}	58.41±1.11^{a}	58.91±1.51^{a}	60.82±0.62^{b}

Values with different superscript letters are significantly different within column.

activity (El-Salam and Mohammed, 2015; Moteetee and Kose, 2017; Mtunzi *et al*., 2017). Phytochemicals such as phenols, tannins, saponins and flavonoids have been identified as active ingredients for this radical scavenging activity (El-Salam and Mohammed, 2015; Moteetee and Kose, 2017; Mtunzi *et al*., 2017). Free radicals and reactive oxygen species cause potentially harmful effects against the biological system, which include damaging DNA, proteins and lipids (Mon *et al*., 2011; Rodrigues *et al*., 2019). Fortunately, these harmful species can be scavenged and neutralised by natural secondary metabolites such as polyphenols, phenolic acids and flavonoids (Array *et al*., 2019; Rodrigues *et al*., 2019).

Table 2. The IC_{50} values of various extracts and fractions from roots and leaves of *S. burchellii*

S/No.	Extracts/Fractions	IC_{50} in µg/mL
1	E1	<200
2	E2	2469.87
3	E3	344.86
4	E4	<200
5	E5	<200
6	E6	623.33
7	E7	2082.63
8	E8	>3000
9	E9	>3000
10	E10	<200
11	E11	292.8
12	E12	>3000
13	Ascorbic Acid	<200

4. Conclusion

Various extracts and their fractions from roots and leaves of *S. burchellii* were evaluated for their DPPH radical scavenging activity. The extracts from roots and their fractions showed radical scavenging activity ranging from 6.60±4.50 to 63.27±1.93%. The extracts from leaves and their fractions showed radical scavenging activity ranging from 3.32±0.95 to 64.91±0.15%. The IC_{50} values of these extracts and fractions were found to be <200 to >3000 µg/mL. Particularly, the methanolic crude extract from roots, ethyl acetate and methanol-water fractions from root extract and ethyl acetate fraction from leaf extract were identified as most potent with the IC_{50} values <200 µg/mL. Based on the percentage inhibition of DPPH radical and IC_{50} value, the ethyl acetate fraction from leaf extract was identified as the most potent among all extracts or fractions. From this study, we concluded that extracts and their fractions from the roots and leaves of *S. burchellii* exhibited promising radical scavenging activity.

Conflict of interests

The authors declare no conflict of interest.

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