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2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts from *Aloiampelos striatula*

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Abstract

Hexane, chloroform, acetone, methanolic and water extracts from leaves and stem-bark of *Aloiampelos striatula* were evaluated for their antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The hexane, chloroform, acetone, methanolic and water extracts from leaves of *A. striatula* showed scavenging activity ranging from 10.30±0.41 - 30.14±1.4, 30.49±0.85 - 45.95±0.22, 38.45±0.49 - 51.65±0.95, 34.83±1.53 - 64.98±0.45 and 54.37±1.24 - 66.74±0.89%, respectively, at various concentrations. The hexane, chloroform, acetone, methanolic and water extracts from stem -bark of *A. striatula* showed scavenging activity ranging from 12.53±3.07 - 29.81±2.43, 17.80±5.17 - 37.20±2.13, 6.15±0.24 - 62.79±3.82, 38.23±1.18 - 67.32±0.55 and 3.16±0.40 - 70.66±0.18%, respectively, at various concentrations. Additionally, the IC_{50} values of these extracts were also determined and were found to be in the range of <200 to >3000 µg/mL. The positive control, vitamin C, showed an IC_{50} value of <200 µg/mL. The therapeutic applications of *A. striatula* are often in traditional Basotho medicine in the Kingdom of Lesotho, which include treating skin problems, high blood pressure, sexually transmitted illnesses, common colds, blood cleansing, stomach ailments and dressing of wounds. Further studies on this plant are required to explore this plant for its commercial applications.

1. Introduction

Aloiampelos striatula belongs to the Asphodelaceae family of the genus *Aloiampelos* (Reynolds, 1950; Van Wyk and Smith, 1996; Seleteng-Kose *et al*., 2015). The vernacular names of this plant are mohalakane and seholobe (Seleteng-Kose *et al*., 2015). *A. striatula* is also known by other names such as *Aloe striatula*, hardy aloe and striped - stemmed aloe. *A. striatula* is distributed throughout the Kingdom of Lesotho and South Africa (Reynolds, 1950). The western region of the Kingdom of Lesotho and Eastern Cape and Free State provinces of South Africa are rich in *A. striatula* (Reynolds, 1950). *A. striatula* has a slender semi-woody stem, grows into a large shrub and reaches up to 2 - meter height. *A. striatula* produces reddish-orange or yellowish flowers (Smith and Van Wyk, 2008). *A. striatula* has dark green curved thin leaves, which are much alike aloe vera leaves. The therapeutic applications of *A. striatula* in the traditional Basotho medicine in the Kingdom of Lesotho (Seleteng-Kose *et al*., 2015) includes treating skin problems, high blood pressure, sexually transmitted illnesses, common colds, blood cleansing, stomach ailments and dressing of wounds (Van Wyk and Gericke, 2000; Moffett, 2010; Seleteng-Kose *et al*., 2015). Particularly, the leaves of *A. striatula* reported having most of the therapeutic potentials (Seleteng-Kose *et al*., 2015). The leaves are soaked in water and consumed by the Basotho for various ailments (Moteetee and Van Wyk, 2011; Moteetee *et al*., 2017). Our literature search showed that this plant has not been studied extensively both phytochemically and pharmacologically. To the best of our knowledge, the antioxidant activity of *A. striatula* has not been reported previously. Therefore, the aim of this study was to evaluate the antioxidant activity of hexane, chloroform acetone, methanolic and water extracts obtained from the leaves and stem-bark of *A. striatula* by DPPH radical scavenging assay. Additionally, the IC_{50} values of these extracts were also determined. This is the first report of this kind from this species gathered from the Kingdom of Lesotho.

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2. Materials and methods

2.1 Plant materials

The leaves and stem-bark of *A. striatula* were collected at Mafikeng village, Roma, Maseru district, The Kingdom of Lesotho, Southern Africa. The plant material was identified by Dr Lerato Seleteng-Kose. Voucher specimen for leaves (AALS) and stem-bark (AASB) were kept in the Organic Chemistry Research Laboratory, Department of Chemistry and Chemical Technology, Faculty of Science and Technology, The National University of Lesotho, Roma campus, The Kingdom of Lesotho.

2.2 Preparation of plant extracts

Approximately, 10kg of leaves and 6kg of stem-bark of *A. striatula* were collected, allowed to air - dry at room temperature separately for six weeks and then ground into fine powder using a laboratory blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 3.5 AMPs, Laboratory and Analytical Supplies). For the current study, a mass of 90.65, 101.55, 203.11, 85.65 and 263.96 g of powdered leaves was taken separately in RB-flasks and was extracted as per the procedure given in the literature (Pillai *et al*., 2019; Matamane *et al*., 2020) with slight modification as detailed below. Approximately, 1 L of hexane, chloroform, acetone, methanol and water were added separately into each RB-flask. The mixtures were macerated for 2 weeks with occasional shaking. The resulting solutions were filtered off separately and the solvents were removed by simple distillation. A mass of 2.24, 10.63, 29.38 and 26.32 g of hexane, chloroform, acetone and methanolic extracts, respectively, were obtained after removal of solvents. However, in the case of water extract, the solvent was removed as much as possible by *vacuo* and the resulting mass was left to stand for two weeks in a fume hood. A mass of 55.47 g of high viscous water extract was obtained and was used for further analysis. Using similar extraction procedures, 1.10, 0.89, 0.75, 0.09 and 2.10 g of hexane, chloroform, acetone, methanolic and water extracts were obtained, respectively from 99.98, 101.34, 104.22, 100.00 and 98.99 g of powdered stem-bark.

2.3 Chemicals used

Hexane, chloroform, methanol, acetone (all solvents were of AR grade, 99.5%), ascorbic acid (AR grade), tris (hydroxymethyl)aminomethane (AR grade) and DPPH (AR grade) were all purchased from Sigma - Aldrich. Deionized water was available at Organic Chemistry Laboratory, NUL, Roma campus, Lesotho.

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

DPPH radical scavenging assay was carried out using a method as described in the literature (Kim *et al*., 2002; Dontha, 2016; Pillai *et al*., 2019; Matamane *et al*., 2020). Briefly, a stock solution of methanolic extract was prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Serial dilutions were made from this stock solution to obtain solutions with concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200 µg/mL. Solutions without extract concentration served as a negative control (Pillai *et al*., 2019). A solution of 3.94 mg of DPPH in 100 mL of methanol served as oxidant, which was prepared just before use and stored in dark to minimize degradation (Pillai *et al*., 2019). A volume of 0.1 mL sample of plant extract solution was mixed with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris - HCL buffer (pH = 7.40) (Pillai *et al*., 2019). Similarly, stock solutions of hexane, chloroform, acetone and water extracts were prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Further dilutions were made from these stock solutions to obtain solutions with concentrations of 3000, 2000, 1500, 1000, 800, 500 and 200 µg/mL (Pillai *et al*., 2019). A volume of 0.1 mL each of this extract was mixed separately with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris - HCL buffer (pH = 7.40). A stock solution of vitamin C (0.3 g) in 50% methanol (v/v) was prepared and serial dilutions were made as previously, which served as a positive control. A volume of 0.1 mL of this solution was mixed with 1.0 mL of 0.1 mM DPPH solution and 0.45mL of 50 mM Tris - HCL buffer (pH = 7.40). The mixtures were incubated for 30 min and the absorbance (optical density) of the mixture was measured at 517 nm using an MRC spectrophotometer (Mode Spectro UV – 11 S/N: UEB 1704020). The following equation was used to calculate the percentage of DPPH radical scavenging activity of extracts.

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

Where A_{test} = Absorbance in the presence of extract or positive control and A_{cont} = Absorbance of negative control (*i.e.* without 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*. The IC_{50} values 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 IC_{50} value for each extract.

Standard deviation was calculated for each concentration from the three values of the experiment.

2.5 Statistical analysis

The statistical data analysis was performed using SPSS Data Editor v16.0 statistics program. The differences were considered statistically significant when $p \leq 0.05$.

3. Results and discussion

Table 1 summarizes the percentage inhibition of DPPH radical scavenging activity of various extracts from leaves and stem-bark of *A. striatula*. A total of ten extracts were prepared. These are i). *A. striatula*-hexane extract from leaves (E1), ii). *A. striatula*-chloroform extract from leaves (E2), iii). *A. striatula*-acetone extract from leaves (E3), iv). *A. striatula*-methanolic extract from leaves (E4), v). *A. striatula*-water extract from leaves (E5), vi). *A. striatula*-hexane extract from stem-bark (E6), vii). *A. striatula*-chloroform extract from stem -bark (E7), viii). *A. striatula*-acetone extract from stem-bark (E8), ix). *A. striatula*-methanolic extract from stem-bark (E9) and x). *A. striatula*-water extract from stem-bark (E10). The extract E1 showed 10.30±0.41, 11.90±2.74, 14.39±0.62, 16.37±1.57, 19.60±2.58, 24.19±6.80 and 30.14±1.40% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The positive control, vitamin C showed 59.86±10.25, 64.16±0.08, 66.12±0.92, 68.14±0.69, 69.31±0.40, 69.56±0.01 and 72.22±0.78% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, E1 showed much lower radical scavenging activity at all concentrations. The extract E2 exhibited 30.49±0.85, 32.28±0.05, 34.59±0.74, 37.43±1.90, 40.79±3.57, 43.94±1.82 and 45.95±0.22% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, E2 showed lower radical scavenging activity at all concentrations. The extract E3 showed 38.45±0.49, 40.84±0.82, 42.89±0.29, 44.60±1.38, 47.10±1.58, 49.26±1.21 and 51.65±0.95% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, E3 also showed lower radical scavenging activity at all concentrations. However, E3 showed relatively higher scavenging activity than E1 and E2 at all concentrations. The extract E4 exhibited 34.83±1.53, 38.12±0.43, 42.82±0.51, 47.04±2.03, 52.77±2.04, 58.30±1.17 and 64.98±0.45% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, E4 also showed lower radical scavenging activity at all concentrations. However, E4 showed comparable scavenging activity as that of E3 at all concentrations. The extract E5 showed 54.37±1.24, 56.44±0.11, 59.25±0.63, 61.22±0.61, 61.85±0.18, 64.45±0.59 and 66.74±0.89% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, E5 showed slightly lower radical scavenging activity at all concentrations. However, E5 showed higher scavenging activity than E4 at all concentrations and showed the highest scavenging activity and most potent among the extracts from the leaves of *A. striatula* (Table 1).

The extract E6 exhibited 12.53±3.07, 15.74±1.85, 17.88±2.79, 19.57±1.19, 21.09±2.17, 23.85±1.41 and 29.81±2.43% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result revealed that E6 showed much lower radical scavenging activity than the positive control, at all concentrations. The extract E7 exhibited 17.80±5.17, 18.82±0.54, 23.41±3.09, 26.58±0.03, 30.74±0.82,

Table 1. The percentage inhibition of DPPH radical by various extracts from leaves and stem-bark of *A. striatula*

Extracts	Concentrations (µg/mL)/Inhibition (%)						
	200	500	800	1000	1500	2000	3000
E1	10.30±0.41^{a}	11.90±2.74^{b}	14.39±0.62^{a}	16.37±1.57^{a}	19.60±2.58^{b}	24.19±6.80^{c}	30.14±1.40^{a}
E2	30.49±0.85^{a}	32.28±0.05^{a}	34.59±0.74^{a}	37.43±1.90^{a}	40.79±3.57^{b}	43.94±1.82^{a}	45.95±0.22^{a}
E3	38.45±0.49^{a}	40.84±0.82^{a}	42.89±0.29^{a}	44.60±1.38^{a}	47.10±1.58^{a}	49.26±1.21^{a}	51.65±0.95^{a}
E4	34.83±1.53^{a}	38.12±0.43^{a}	42.82±0.51^{a}	47.04±2.03^{a}	52.77±2.04^{a}	58.30±1.17^{a}	64.98±0.45^{a}
E5	54.37±1.24^{a}	56.44±0.11^{a}	59.25±0.63^{a}	61.22±0.61^{a}	61.85±0.18^{a}	64.45±0.59^{a}	66.74±0.89^{a}
E6	12.53±3.07^{b}	15.74±1.85^{a}	17.88±2.79^{b}	19.57±1.19^{a}	21.09±2.17^{a}	23.85±1.41^{a}	29.81±2.43^{a}
E7	17.80±5.17^{c}	18.82±0.54^{a}	23.41±3.09a	26.58±0.03^{a}	30.74±0.82^{a}	34.91±0.00	37.20±2.13^{a}
E8	6.15±0.24^{a}	13.17±1.82^{a}	27.66±0.41^{a}	33.37±0.08^{a}	35.67±0.29^{a}	48.62±2.09^{a}	62.79±3.82^{b}
E9	38.23±1.18^{a}	41.20±0.21^{a}	42.28±1.14^{a}	46.05±0.10^{a}	48.65±0.71^{a}	51.08±0.38^{a}	67.32±0.55^{a}
E10	3.16±0.40^{a}	14.34±2.61^{b}	18.36±1.54^{a}	52.22±1.27^{a}	59.11±1.06^{a}	65.13±2.03^{a}	70.66±0.18^{a}
Vitamin C	59.86±10.25^{e}	64.16±0.08^{a}	66.12±0.92^{a}	68.14±0.69^{a}	69.31±0.40^{a}	69.56±0.01^{a}	72.22±0.78^{a}

Values with different superscript letters are significantly different.

34.91±0.00 and 37.20±2.13% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Therefore, E7 showed lower scavenging activity than the positive control. The extract E8 exhibited 6.15±0.24, 13.17±1.82, 27.66±0.41, 33.37±0.08, 35.67±0.29, 48.62±2.09 and 62.79±3.82% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result indicated that at lower concentrations, E8 showed much lower scavenging activity compared to the positive control. However, at higher concentrations, the scavenging activity of E8 was very significant. It means that at higher concentrations, E8 might have rich phytochemicals such as polyphenols, phenolic acids, flavonoids etc. in sufficient quantities. These phytochemicals have been reported as important classes of antioxidants in natural sources (Array *et al*., 2019; Rodrigues *et al*., 2019). The extract E9 exhibited 38.23±1.18, 41.20±0.21, 42.28±1.14, 46.05±0.10, 48.65±0.71, 51.08±0.38 and 67.32±0.55% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. The extract E9 showed lower scavenging activity at all concentrations relative to the positive control. However, E9 showed significant scavenging activity at each concentration and much higher scavenging activity than E8. The extract E10 showed 3.16±0.40, 14.34±2.61, 18.36±1.54, 52.22±1.27, 59.11±1.06, 65.13±2.03 and 70.66±0.18% of inhibition at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result indicated that at lower concentrations, E10 showed much lower scavenging activity than the positive control. However, at higher concentrations at 1000, 1500, 2000 and 3000 µg/mL, it showed significant scavenging activity and showed comparable scavenging activity as that of positive control (Table 1). Again, at higher concentrations, E10 might have contained scavenging rich phytochemicals such as polyphenols, phenolic acids, flavonoids etc. in sufficient quantities.

Additionally, the IC_{50} values of these ten extracts were also determined and the results are summarized in Table 2. Extracts E1 - E10 showed IC_{50} values of >3000, ~2300, ~1400, ~1200, <200, >3000, >3000, ~2200, ~1660 and >3000 µg/mL, respectively (Table 2). The positive control, vitamin C, showed an IC_{50} value of <200 µg/mL (Table 2). The extract E5 was found to be most potent with an IC_{50} value of <200 µg/mL and this value was similar to the positive control.

The water extract from leaves of *A. striatula* (E5) was the most potent among all ten extracts. The extracts E2, E3, E4 and E9 showed significant scavenging activity at all concentrations. The extracts E8 and E10 showed lower scavenging activity at lower concentrations but at higher concentrations, both they exhibited significant activities. As stated previously, at higher concentrations, E8 and E10 might have contained scavenging rich phytochemicals such as polyphenols, phenolics, flavonoids etc. in sufficient quantities. The extracts E1, E6 and E7 showed weak to moderate scavenging activity at all concentrations and showed relatively lower scavenging activity among all ten extracts. The leaves of *A. striatula* reported having many therapeutic applications (Van Wyk and Gericke, 2000; Moffett, 2010; Seleteng-Kose *et al*., 2015). However, the therapeutic applications of stem-bark and root of *A. striatula* have not been reported, previously. This study also confirmed that the extracts from the leaves of *A. striatula* showed higher scavenging activity than the extracts from stem-bark. Particularly, the water extract from leaves of *A. striatula* (E5) showed the highest scavenging activity among all ten extracts and identified as the potent extract.

Table 2. IC_{50} values for various extracts from leaves and stem -bark of *A. striatula*

S. No	Extracts	IC_{50} in µg/mL
1	E1	>3000
2	E2	~2300
2	E3	~1400
4	E4	~1200
5	E5	<200
6	E6	>3000
7	F7	>3000
8	E8	~2200
9	E9	~1660
10	E10	>3000
11	Vitamin C	<200

4. Conclusion

In this study, hexane, chloroform, acetone, methanolic and water extracts from leaves and stem-bark of *A. striatula* were evaluated for their antioxidant activity by DPPH radical scavenging assay. Their radical scavenging activity at various concentrations was found to be in the range of 6.15±0.24 to 70.66±0.18%. Additionally, the IC_{50} values of these extracts were also determined and were found to be in the range of <200 to >3000 µg/mL. The positive control, vitamin C, showed an IC_{50} value of <200 µg/mL. The water extract from leaves of *A. striatula* showed similar IC_{50} value as that of positive control. *i.e.* <200 µg/mL. This water extract from leaves of *A. striatula* finds therapeutic applications in the traditional Basotho medicine in the Kingdom of Lesotho. Our study also confirmed this fact that the water extract from leaves of *A. striatula* showed the highest scavenging potential and identified as the most potent among all ten extracts. This is the first report of this kind from this species collected from the Kingdom

of Lesotho. Further studies on this plant will be useful to explore for any commercial applications.

Conflict of interests

The authors declare no conflict of interest.

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