Vol. 13(15), pp. 329-334, August, 2019
DOI: 10.5897/JMPR2019. 6793
Article Number: 6639C1861615
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

DPPH radical scavenging activity of extracts from *Rhamnus prinoides*

Manoharan Karuppiah Pillai[1]*, Lehlohonolo Isaac Santi[1] and Sibusisiwe Magama[2]

[1]Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, South Africa.
[2]Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, South Africa.

Received 24 May, 2019; Accepted 5 July, 2019

Hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *Rhamnus prinoides* were evaluated for their antioxidant activity by DPPH radical scavenging assay. The leaves extracts showed scavenging activity ranging from 03.33±0.89 to 55.03±3.40 µg mL^{-1} while the stem-bark extracts showed relatively strong scavenging activity ranging from 03.65±1.02 to 59.55±2.27 µg mL^{-1}. The IC_{50} values of *R. prinoides* hexane leaves extract (RPHELS), *R. prinoides* chloroform leaves extract (RPCHLS), *R. prinoides* ethyl acetate leaves extract (RPEALS), *R. prinoides* methanolic leaves extract (RPMELS), *R. prinoides* hexane stem-bark extract (RPHESB), *R. prinoides* chloroform stem-bark extract (RPCHSB), *R. prinoides* ethyl acetate stem-bark extract (RPEASB) and *R. prinoides* methanolic stem-bark extract (RPMESB) were found to be >3000, >3000, >3000, 950.42, ~1500, 710.50, ~1000 and 902.78 µg mL^{-1}, respectively. The positive control ascorbic acid showed an IC_{50} value of <200 µg mL^{-1}. From this study, we concluded that the extracts from *R. prinoides* showed promising antioxidant activity. *R. prinoides* finds therapeutic applications in the traditional medicine. Further research is required to commercialize products from this plant.

Key words: Antioxidant, ascorbic acid, *Rhamnus prinoides*, radical scavenging assay, methanolic extract, chloroform extract, hexane extract, ethyl acetate extract.

INTRODUCTION

Rhamnus prinoides belongs to the Rhamnaceae family (Dale, 2000; Dlamini and Turner, 2002). *R. prinoides* is also known by other names such as African Dogwood, Glossy-leaf and mififi. *R. prinoides* is widely distributed in East and South African countries (Alemu et al., 2007; Abegaz et al., 1999) which include Ethiopia, Botswana, Eritrea, Lesotho, Namibia, South Africa, Swaziland, Uganda and Kenya (Ashine, 2015). *R. prinoides* grows up to 4.5-m height and found in evergreen forests, in the wild and along water streams. Although, *R. prinoides* is a slow growing plant in low rainfall areas, it can grow 1 m per annum in wet areas (Ferede et al., 2018). The leaves begin with pale green and turn to dark and shiny on maturation. The roundish red berries attract bees and

*Corresponding author. E-mail: kmharan@rediffmail.com. Tel: +266 5221 3499.

domestic fowl. *R. prinoides* casts a very deep shade such that it will not allow other plants to grow around it. *R. prinoides* flowers towards the end of the year and fruiting occurs at the beginning of the year. *R. prinoides* finds therapeutic applications in the traditional medicine. The decoction of roots has been used to treat pulmonary tuberculosis, pneumonia, bladder and kidney problems (Maliehe, 1997; Van Wyk and Moteetee, 2011). The bark has been used to induce vomiting. An extract of the root together with the bark of *Erythrina tomentosa* has been used to relief colic. The leaves have been applied as a liniment to simple sprains. *R. prinoides* has been used to provide a special aroma and flavor (Shale and Gashe, 1991; Abegaz et al., 1999). *R. prinoides* has also been used in the beer industry as a hopping agent. Geshoidin, a naphathalenic glycoside, present in the stem-bark is responsible for providing bitterness in alcoholic beverages (Nindi et al., 1999). The antioxidant activity of 97% ethanolic extracts from leaves of *R. prinoides* has been reported previously (Ashine, 2015). Methanolic and aqueous extracts from roots of *R. prinoides* have also evaluated for their DPPH radical scavenging activity (Kimondo et al., 2019). However, to the best of our knowledge, the scavenging activity of hexane, chloroform, ethyl acetate and methanolic extracts of the leaves and stem-bark of *R. prinoides* has not been reported previously, particularly the plant species gathered from the Kingdom of Lesotho. The aim of the present study was to evaluate the antioxidant activity of these extracts by DPPH radical scavenging assay and to determine their IC_{50} values. The results obtained are communicated in this article.

MATERIALS AND METHODS

Plant materials

The leaves and stem-bark of *R. prinoides* were collected from the foothills of Popa and Popanyane Mountains at Mokhokhong village, Roma, Maseru district, the Kingdom of Lesotho, Southern Africa in January 2019. A voucher specimen viz. Santi/RPLS/2019 for leaves and Santi/RPSB/2019 for stem-bark were kept separately in the Organic Research Laboratory, Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, Maseru district, Kingdom of Lesotho, Southern Africa.

Processing of materials

The leaves were allowed to air dry at room temperature for two weeks and then ground into powder using a commercial blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 50-80 Hz, 3.6AMPs, Laboratory and Analytical Supplies). The chopped stem-barks were allowed to air dry at room temperature for two weeks and ground into powder using the blender.

Preparation of plant extracts

The powdered leaves (300.043 g) of *R. prinoides* were extracted with methanol under cold conditions for 3 days. The solution was filtered using a filter paper (Boeco, Germany). The solvent was removed by *vacuo* and the same procedure was repeated once again. Finally, the plant material was extracted with hot methanol. 40.1858 g of combined methanol extract was obtained after removal of solvent. The same extraction procedure was followed to get hexane (3.2274 g), chloroform (10.6285 g) and ethyl acetate (11.4763 g) extracts from 300.254, 300.131 and 299.921 g of powdered leaves, respectively. The powdered stem-bark (299.530 g) of *R. prinoides* was extracted first with methanol at room temperature for 3 days followed by a reflux condition for 6 h. 32.2047 g of combined crude methanol extract was obtained after removal of solvent. The same extraction procedures were followed to get hexane (2.5895 g), chloroform (5.4327 g) and ethyl acetate (8.1493 g) extracts from 300.014, 300.157, and 300.422 g of powdered stem-bark, respectively.

Chemicals and solvents used

Ascorbic acid, DPPH, hexane (AR grade, 99.5%), chloroform (AR grade, 99.5%), ethyl acetate (AR grade, 99.5%) and methanol (AR grade, 99.5%) were all purchased from Sigma-Aldrich.

Antioxidant activity

The antioxidant activity of the extracts was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described in literature (Kim et al., 2002). Briefly, stock solution of the 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^{-1}. Solutions without extract concentration served as negative control. 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. 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 (p H7.40). Similarly, stock solutions of hexane, chloroform and ethyl acetate 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, 200 and 0 µg mL^{-1}. 0.1 mL each of extract was mixed separately with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of 50 mM Tris-HCL buffer (pH7.40). A stock solution of ascorbic acid (0.3 g) in 50% methanol (v/v) was prepared and serial dilutions were made as previously and served as positive control. 0.1 mL 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). The mixtures were incubated for 30 min and their optical density was measured at 517 nm. Percentage inhibition of DPPH free radical was calculated using the equation:

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

where A_{test} = Absorbance in the presence of extract or positive control and A_{cont} = Absorbance of negative control.

The IC_{50} value is defined as the concentration (in µg mL^{-1}) 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. The IC_{50} values were calculated from graphs by plotting extract concentrations vs. percentage inhibition of DPPH radical using Microsoft Excel. Each experiment was carried out in triplicate and the averages of the three values were used to calculate IC_{50} values. Standard deviation was calculated for each concentration from the three values of the experiment.

Statistical analysis

Results were expressed as means of three determinations. One-way analysis of variance (ANOVA) was used to compare means at the significance level $p < 0.05$. All analysis was performed by Microsoft Excel software.

RESULTS AND DISCUSSION

Table 1 summarizes the DPPH radical scavenging activity of hexane, chloroform, ethyl acetate and methanolic extracts of the leaves and stem-bark of *R. prinoides*. *R. prinoides* hexane leaves extract (RPHELS) showed 3.33±0.89, 3.80±2.60, 8.61±1.39, 10.12±0.84, 13.39±2.94, 20.10±3.23 and 34.56±6.51% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. The positive control, ascorbic acid, showed 53.01±3.98, 53.46±0.14, 53.51±0.77, 53.82±0.54, 54.12±1.64, 54.34±0.92 and 56.45±5.45% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. These results revealed that RPHELS showed very weak radical scavenging activity relative to positive control at all concentrations. *R. prinoides* chloroform leaves extracts (RPCHLS) showed 23.01±3.44. 27.15±5.18, 30.35±1.02, 31.38±0.11, 47.37±4.14, 48.38±4.15 and 48.49±3.17% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. This result showed that RPCHLS has lower activity than positive control at all concentrations. *R. prinoides* ethyl acetate leaves extract (RPEALS) showed 17.71±1.02, 17.96±0.82, 19.48±1.50, 28.51±0.62, 36.05±6.26, 38.94±5.75 and 42.33±5.88% of scavenging activity at 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. This result revealed that RPEALS exhibited weak activity at low concentrations relative to positive control. However, at high concentration of 3000 µg/mL, it showed higher scavenging activity of 42.33±5.88%. *R. prinoides* methanolic leaves extract (RPMELS) showed 21.01±3.80, 33.74±1.42, 41.50±2.73, 50.93±5.27, 52.93±2.88, 54.34±2.70 and 55.03±3.40% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. This result revealed that RPMELS exhibited weak activity at low concentrations relative to positive control. However, RPMELS has comparable activity as that of positive control at high concentrations.

R. prinoides hexane stem-bark extract (RPHESB) showed 12.39±3.19, 18.93±1.04, 30.47±0.82, 42.15±4.06, 50.24±1.50, 51.52±2.47 and 52.15±1.06% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. This result revealed that RPHESB exhibited weak activity at low concentrations relative to positive control. However, at higher concentrations such as 1500, 2000 and 3000 µg/mL, RPHESB exhibited comparable activity as that of positive control. *R. prinoides* chloroform stem-bark extract (RPCHSB) showed 42.37±5.65, 43.30±2.98, 52.23±2.46, 53.64±4.42, 54.06±1.41, 54.41±2.17 and 55.22±2.48% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. Thus, RPCHSB exhibited comparable activity as that of positive control at all concentrations except at concentrations of 200 and 500 µg mL^{-1}. RPCHSB showed 42.37±5.65 and 43.30±2.98% of scavenging at concentrations 200 and 500 µg/mL, respectively, while the positive control showed 53.01±3.98 and 53.46±0.14% of scavenging activity at concentrations 200 and 500 µg mL^{-1}, respectively. *R. prinoides* ethyl acetate stem-bark extract (RPEASB) showed 3.65±1.02, 40.04±1.50, 47.09±4.36, 50.31±7.80, 51.61±2.27, 54.23±1.83 and 57.43±3.28% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Thus, RPEASB exhibited very weak activity relative to positive control at low concentration of 200 µg mL^{-1}. *R. prinoides* methanolic stem-bark extract (RPMESB) showed 30.09±5.26, 41.69±2.27, 45.80±2.54, 52.78±6.43, 55.37±3.90, 56.19±3.58 and 59.55±2.27% of scavenging activity at concentrations 200, 500, 800, 1000, 1500, 2000 and 3000 µg mL^{-1}, respectively. This result revealed that RPMESB exhibited weak activity at low concentrations relative to positive control. However, at high concentrations such as 2000 and 3000 µg/mL, it showed higher scavenging activity of 56.19±3.58 and 59.55±2.27%, respectively. Among the extracts (RPHELS, RPCHLS, RPEALS, RPMELS, RPHESB, RPCHSB, RPEASB and RPMESB) from *R. prinoides*, RPMESB showed the highest scavenging activity (Table 1). For comparison and clarity, the percentage of scavenging activity of these extracts at various concentrationsare is as shown in Figures 1 and 2. The IC_{50} values of hexane, chloroform, ethyl acetate and methanolic extracts of the leaves and stem-bark of *R. prinoides* are shown in Table 2. RPHELS, RPCHLS, RPEALS, RPMELS, RPHESB, RPCHSB, RPEASB and RPMESB exhibited IC_{50} values of >3000, >3000, >3000, 950.42, ~1500, 710.50~1000 and 902.78 µg mL^{-1}, respectively. RPCHSB is the most potent with IC_{50} value of 710.50 µg mL^{-1}. The positive control ascorbic acid showed an IC_{50} value of <200 µg mL^{-1}.

The DPPH radical scavenging activity of 97% ethanolic extract from leaves of *R. prinoides* has previously been reported and its maximum radical scavenging was found to be 81.148% at a concentration of 24 mg mL^{-1} (Ashine, 2015) and the IC_{50} value was determined to be 5.2 mg mL^{-1}. The positive control, ascorbic acid, showed 93.77% scavenging activity with an IC_{50} value of 0.24 mg mL^{-1} in the same assay (Ashine, 2015). Therefore, when compared with the present study on the hexane, chloroform, ethyl acetate and methanolic extracts from leaves of *R. prinoides*, this 97% ethanolic extract from leaves of *R. prinoides* showed higher radical scavenging activity and lower IC_{50} value. This 97% ethanol might have more extractive power of active constituents than

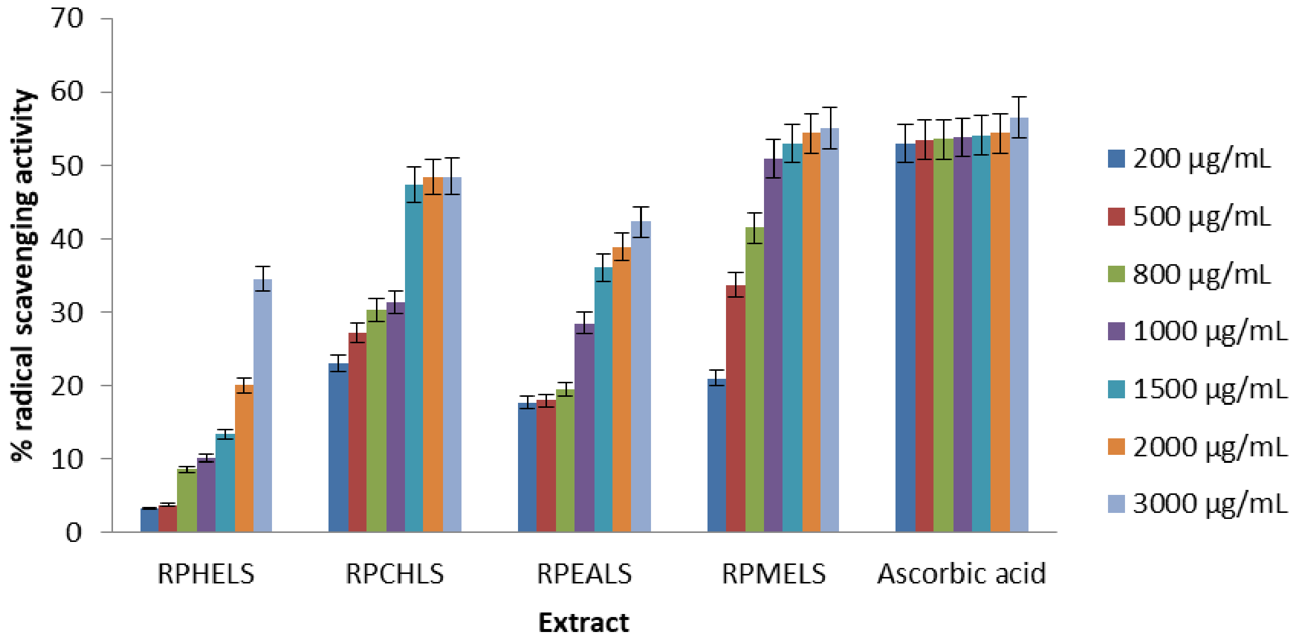

Figure 1. Percentage of radical scavenging of leaves extracts and ascorbic acid at various concentrations. RPHELS: *R. Prinoides* hexane leaves extract; RPCHLS: *R. prinoides* chloroform leaves extract; RPEALS: *R. prinoides* ethyl acetate leaves extract; RPMELS: *R. prinoides* methanolic leaves extract; Ascorbic acid in 50% methanol served as positive control.

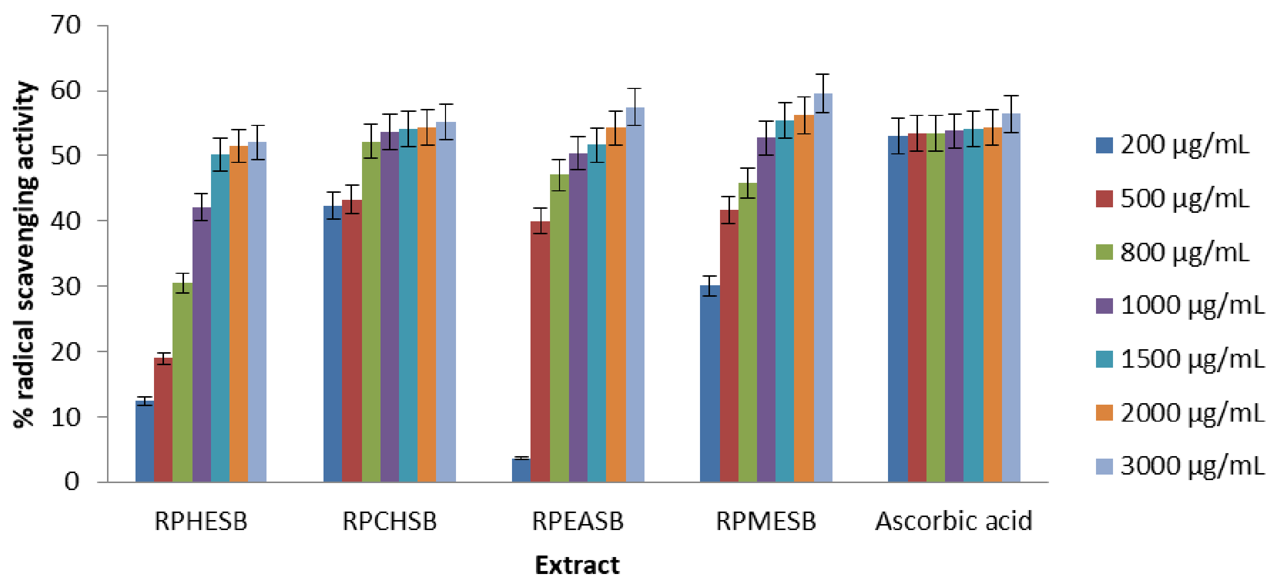

Figure 2. Percentage of radical scavenging of stem-bark extracts and ascorbic acid at various concentrations. RPHESB: *R. prinoides* hexane stem-bark extract; RPCHSB: *R. prinoides* chloroform stem-bark extract; RPEASB: *R. prinoides* ethyl acetate stem-bark extract; RPMESB: *R. prinoides* methanolic stem-bark extract; Ascorbic acid in 50% methanol served as positive control.

the solvents used in the present study. Additionally, the collection of plant materials at different geographic locations will also play a vital role in determining the active constituents of extracts. Methanolic and aqueous roots extracts from *R. prinoides* have also been screened for their DPPH radical scavenging activity. Their IC_{50}

Table 1. Percentage of radical scavenging activity of extracts from *R. prinoides* at various concentrations.

Extract	Concentrations (μg mL^{-1})/ Percentage of inhibition						
	200	500	800	1000	1500	2000	3000
RPHELS	03.33±0.89	03.80±0.26	08.61±1.39	10.12±0.84	13.39±2.94	20.10±3.23	34.56±6.51
RPCHLS	23.01±3.44	27.15±5.18	30.35±1.02	31.38±0.11	47.37±4.14	48.38±4.15	48.49±3.17
RPEALS	17.71±1.02	17.96±0.82	19.48±1.50	28.51±0.62	36.05±6.26	38.94±5.75	42.33±5.88
RPMELS	21.01±3.80	33.74±1.42	41.50±2.73	50.93±5.27	52.93±2.88	54.34±2.70	55.03±3.40
RPHESB	12.39±3.19	18.93±1.04	30.47±0.82	42.15±4.06	50.24±1.50	51.52±2.74	52.15±1.06
RPCHSB	42.37±5.65	43.30±2.98	52.23±2.46	53.64±4.42	54.06±1.41	54.41±2.17	55.22±2.48
RPEASB	03.65±1.02	40.04±1.50	47.09±4.36	50.31±7.80	51.61±2.27	54.23±1.83	57.43±3.28
RPMESB	30.09±5.26	41.69±2.27	45.80±2.54	52.78±6.43	55.37±3.90	56.19±3.58	59.55±2.27
Asc. acid	53.01±3.98	53.46±0.14	53.51±0.77	53.82±0.53	54.12±1.64	54.34±0.92	56.45±5.45

RPHELS: *R. prinoides* hexane leaves extract; RPCHLS: *R. prinoides* chloroform leaves extract; RPEALS: *R. prinoides* ethyl acetate leaves extract; RPMELS: *R. prinoides* methanolic leaves extract; RPHESB: *R. prinoides* hexane stem-bark extract; RPCHSB: *R. prinoides* chloroform stem-bark extract; RPEASB: *R. prinoides* ethyl acetate stem-bark extract; RPMESB: *R. prinoides* methanolic stem-bark extract; Asc. Acid = Ascorbic acid in 50 % methanol served as positive control. All experiments were conducted in triplicate (n=3) and reported as the mean of three values together with standard deviation, ±SD.

Table 2. The IC_{50}values of various extracts of *R. prinoides*by DPPH radical scavenging assay.

S/N	Extract	IC_{50} (μg mL^{-1})
1	RPHELS	>3000
2	RPCHLS	>3000
3	RPEALS	>3000
4	RPMELS	950.23
5	RPHESB	~1500
6	RPCHSB	710.50
7	RPEASB	~1000
8	RPMESB	902.78
9	Asc. acid	<200

RPHELS: *R. prinoides* hexane leaves extract; RPCHLS: *R. prinoides* chloroform leaves extract; RPEALS: *R. prinoides* ethyl acetate leaves extract;RPMELS: *R. prinoides* methanolic leaves extract; RPHESB: *R. prinoides* hexane stem-bark extract; RPCHSB: *R. prinoides* chloroform stem-bark extract; RPEASB: *R. prinoides* ethyl acetate stem-bark extract; RPMESB: *R. prinoides* methanolic stem-bark extract; Asc. Acid = Ascorbic acid in 50 % methanol served as positive control. All experiments were conducted in triplicate (n=3) and reported as the mean of three values together with standard deviation, ±SD.

values were found to be 377.27 and ~250 μg mL^{-1}, respectively (Kimondo et al., 2019). The positive control, ascorbic acid, showed an IC_{50} value 50.32 μg mL^{-1} in the same assay (Kimondo et al., 2019). Additionally, the kinetics of acetylcholinesterase (AChE) inhibitory activity of aqueous extract from *R. prinoides* has previously been reported (Catherine and Edward, 2009). The IC_{50} value for *R. prinoides* was found to be 0.201 mg mL^{-1}. The AChE inhibitory activity of *R. prinoides* was found to be higher than that of some Portugese and Danish medicinal plants (Ferreira et al., 2006; Adersen et al., 2006). Biologically important secondary metabolites such as emodin, physcion, prinoidin, rhamnazin, geshodin and many other emodin-derived compounds have been reported from *R. prinoides* (Van Staden and Drewes, 1994; Abegaz and Kebete, 1995). Alkaloids, flavonoids, terpenoids, anthraquinones, saponins, polyphenols, etc., classes of compounds have also been reported from various extracts of *R. prinoides* (Molla et al., 2016).

Conclusion

DPPH radical scavenging activity of hexane, chloroform, ethyl acetate and methanolic extracts from leaves and stem-bark of *R. prinoides* collected from the Kingdom of

Lesotho have been evaluated. The leaves extracts showed scavenging activity ranging from 03.33±0.89 to 55.03±3.40 µg mL^{-1} while the stem-bark extracts showed relatively strong scavenging activity ranging from 03.65±1.02 to 59.55±2.27 µg mL^{-1}. The IC_{50} values of these extracts were also determined and were found to be in the range of 710.50 to >3000 µg mL^{-}1. *R. prinoides* finds therapeutic applications in the traditional medicine and showed promising antioxidant activity. Therefore, further studies will be useful to commercialize products from this plant.

CONFLICT OF INTERESTS

The authors declared no conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge the National University of Lesotho for its overall support.

REFERENCES

Abegaz B, Kebete T (1995). Geshodin: Abitter principle of Rhamnusprinoides and other constituents of the leaves. Bulletin of Chemical Society of Ethiopia 9(2):107-114.

Abegaz BM, Ngadjui BT, Bezabih M, Modee, LK (1999). Novel natural products from marketed plants of eastern and Southern Africa. Pure and Applied Chemistry 71(6):919-926.

Adersen A, Gauguin B, Gudiksen L, Jager AK (2006). Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology 104(3):418-422.

Alemu H, Abegaz BM, Bezabih M (2007). Electrochemical Behaviour and Voltammetric Determination of Geshodin and its Spectrophotometric and Antioxidant properties in aquoues buffer solutions. Bulletin of Chemical Society of Ethiopia 21(2):189-204.

Ashine F (2015). Production of barley malt beer using Gesho (Rhamnusprinoides) in Ethiopia. School of Chemical and Bioengineering, Addis Ababa, Ethiopia. pp. 18-33.

Catherine MC, Edward JO (2009). Kinetics of acetylcholinesterase inhibition activities by aqueous extracts of *Acacia nilotica* (L.) and Rhamnusprinoides (L'Hér.). African Journal of Pharmacy and Pharmacology 3(10):469-475.

Dale N (2000). Flowering plants of the Santa Monica Mountains, Capra Press, 2nd Ed. P 166.

Dlamini MD, Turner S (2002). Rhamnus prinoides L'Hérit. Witwatersrand National Botanical Garden, South African National Biodiversity Institute, South Africa P 1.

Ferede T, Amare D, Endalamaw B, Genanew T (2018). Rhamnus prinoides in Noeth West Ethiopia: Production, contribution and constraints. Recent Advances in Petrochemical Sciences 4(5):001-006.

Ferreira A, Proenca C, Serralheiro MLM, Araujo MEM (2006). The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. Journal of Ethnopharmacology 108(1):31-37.

Kim JK, Noh JH, Lee S, Choi JS, Suh H, Chung HY, Song YO, Choi WC (2002). The first total synthesis of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) and its antioxidant activity. Bulletin of Korean Chemical Society 23(5):661-662.

Kimondo J, Mutal P, Njogu P, Kimwele C (2019). Evaluation of the Antioxidant Activity of Nine Plants Used Medicinally by the IlkisonkoMaasai Community of Kenya. Free Radicals and Antioxidants 9(1):29-34.

Maliehe EB (1997). Medicinal plants and herbs of Lesotho. Maseru, Mafeteng Development Project pp. 40-49.

Molla Y, Nedi T, Tadesse G, Alemayehu H, Shebeshi W (2016). Evaluation of the in vitro antibacterial activity of the solvent fractions of the leaves of *Rhamnusprinoides*L'Herit (Rhamnaceae) against pathogenic bacteria. BMC Complementary and Alternative Medicine 16:287-296.

Moyo M, Amoo SO, Ncube BM, Ndhlala AR, Finnie JF, Van Staden J (2013). Phytochemical andantioxidant properties of unconventional leafy vegetables consumed insouthern Africa. South African Journal of Botany 84:65-71.

Ndhlala AR, Aderogba MA, Ncube B, Van Staden J (2013). Anti-Oxidative and Cholinesterase Inhibitory Effects of Leaf Extracts and their Isolated Compounds from Two closely related croton species. Molecules 18(2):1916-1932.

Nindi MM, Kgarebe BV, Wolfender JL, Abegaz BM (1999). Electrospray liquid chromatography- mass spectrometry of the leaf extract of *Rhamnusprinoides*. Phytochemchemical Analalysis 10(2):69-75.

Shale S, Gashe BA (1991). The microbiology of Tella fermentation. Sinet Ethiopia Journal of Sciences 14:81-92.

Van Staden LF, Drewes SE (1994). Knipholone from Bulbinelatifolia and Bulbinefrutescens. Phytochemistry 35(3):685-686.

Van Wyk BE, Moteetee A (2011). The medical ethnobotany of Lesotho: A Review. Bothalia 41(1):209-228.