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Antioxidant activity of extracts from *Searsia dentata*

Seroke Molefi and Manoharan Karuppiah Pillai

Abstract
Searsia dentata has been used in the treatment of epilepsy and convulsions. The aim of the present study was to evaluate the antioxidant activity of hexane, chloroform, ethyl acetate and methanolic extracts obtained separately from leaves and stem-bark of *S. dentata*. Maceration and hot solvent extractions techniques were used to obtain these extracts. DPPH and ferric reducing power assays, Folin-Ciocalteu and colorimetric aluminium chloride methods were used in this study. The DPPH radical scavenging activity and ferric reducing power of these extracts were found to be in the range of 7.34±1.09 to 77.84±8.53% and 0.054±0.002 to 1.956±0.037 at 700nm, respectively. The TPCs and TFCs of these extracts were determined to be in the range of 33.590±1.702 to 657.72±0.500mgTAE/g of dry weight of extract and 5.61±0.037 to 59.08±0.371mgQE/g of dry weight of extract, respectively. From this study, we concluded that *S. dentata* could be a valuable source of antioxidants.

Keywords: *Searsia dentata*, Anacardiceae, DPPH assay, ferric reducing power, total phenolic contents (TPCs), total flavonoid contents (TFCs)

1. Introduction
Searsia dentata belongs to the Anacardiceae family of Searsia (Rhus) genus [1-3]. *S. dentata* is also known by vernacular names such as Mabele-bele, Nana berry and Nana Bessie [3, 4]. *S. dentata* is commonly found in rocky ravines, woodland and along watercourses in the Kingdom of Lesotho and South Africa [5]. *S. dentata* is a deciduous shrub or small tree and grows up to 5 meters height. *S. dentata* has smooth greyish brown bark. The leaves are green and turn into yellow to orange- reddish on maturation. The flowers are creamy white and yield orange-reddish drupes of 5-6mm diameter, which turn into brown on drying. The ripe fruits are served as a good source of diet for fruit eating birds [6]. *S. dentata* has been used in the treatment of epilepsy and convulsions[7]. Many species from the Searsia genus have been exhibited a variety of biological and pharmacological activities, which include anti-inflammatory, anticancer, antimalarial, antimicrobial, antidiarrheal, antiviral and antioxidant activities [3]. The objective of the present study was to evaluate 2,2-diphenyl-1-picrylhydrazil radical (DPPH) scavenging activity and ferric reducing power and to determine the total phenolic contents (TPCs) and total flavonoid contents (TFCs) of various extracts obtained from the leaves and stem-bark of *S. dentata*. Additionally, the IC_{50} values of these extracts were also determined using DPPH assay. The results are communicated in this article. To the best of our knowledge, this is the first report of this kind especially the species collected from the Kingdom of Lesotho.

2. Materials and Methods
2.1 Chemicals and reagents
The following analytical reagent (AR) grade of solvents, chemicals and reagents were used in this study. Methyl alcohol, ethyl acetate, hexane, acetone, chloroform, Folin-Ciocalteu reagent, tris-(hydroxymethyl) aminomethane, DPPH and tannic acid (all from Sigma-Aldrich). Sodium hydroxide, sodium dihydrogen phosphate and disodium hydrogen carbonate (all from Minema Ltd), L-ascorbic acid (Associated Chemical Enterprises or Minema Ltd), quercetin (Acros Organics), potassium ferricyanide (Holpro Analytics Pty Ltd), ferric chloride and trichloroacetic acid (both from BDH Chemicals Ltd), sodium nitrite (Unilab) and sodium carbonate (Radchem Laboratory Supplies).

2.2 Plant materials
The leaves and stem-bark of *S. dentata* were collected along the Roma valley, Roma, Maseru district of the Kingdom of Lesotho. The plant materials were identified by Dr Seleteng-Kose, Department of Biology, Roma campus, National University of Lesotho (NUL). A voucher specimen viz. Seroke/SDLS/02/2021 and Seroke/SDSB/02/2021 were deposited at the Organic Chemistry Laboratory, NUL, respectively for the leaves and stem-bark of *S. dentata*

2.3 Processing of plant materials
The plant materials were allowed to air-dry at the Organic Chemistry Laboratory, NUL for four weeks. With a help of a laboratory-scale grinding machine (Waring Blender, 80119, Model HGB2WT93, 240V AC, 3.5 AMPs), the leaves and stem-bark were separately ground into powder. A mass of 3.562 and 3.465kg of powdered leaves stem-bark respectively were obtained.

2.4 Preparation of plant extracts
Approximately, 721g of powdered leaves was macerated with hexane (2L) at room temperature for three days. The solution was decanted, filtered and the solvent was removed under vacuum. A hexane leaf crude extract thus obtained was kept in a beaker. The same procedure was repeated once again with the same plant material and the hexane leaf crude extract was combined together. Finally, the same plant material was refluxed with hexane for 24 hours. The filtrate was concentrated under vacuum and the hexane leaf crude extract thus obtained was combined with the previously obtained hexane leaf crude extract. A total mass of 19.647g of brown coloured combined hexane leaf crude extract was obtained. Following similar extraction procedure, 36.841, 14.683, 38.981 and 59.756g of brown to dark brown coloured combined crude extracts of chloroform, ethyl acetate, acetone and methanol were obtained, respectively from 687, 754, 715 and 623g of powdered leaves. Similarly, a mass of 2.893, 4.851, 8.257, 9.378 and 3.477g of brown to dark brown coloured combined stem-bark crude extracts of hexane, chloroform, ethyl acetate, acetone and methanol were obtained respectively from 689, 673, 711, 691 and 701g of powdered stem-bark.

2.5 Evaluation of radical scavenging activity and determination of their IC_{50} values
DPPH radical scavenging activity of various extracts obtained from the leaves and stem-bark of *S. dentata* was evaluated by the methods outlined in the literature [8-11]. A stock solution of each extract was prepared separately by dissolving 30mg of extract in 10mL of 50% methanol (v/v in water). Further dilutions such as 200, 500, 800, 1000, 1500, 2000 and 3000µg/mL were prepared from each of this stock solution and followed the rest of the procedure as per literature [8-11]. The absorbance of the resulting mixture was measured at 517nm using MRM, Mode Spectro UV-11, S/N: UEB 1704200 Spectrophotometer. The percent inhibition of DPPH radical scavenging activity was calculated using the formula given below.

$$\text{DPPH scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where, A_{cont} = Absorbance of negative control (*i.e.* without extract) and A_{test} = Absorbance in the presence of extract or positive control ascorbic acid.

Additionally, the IC_{50} values of these extracts were determined from the linear regression equation by plotting various concentrations of extract (in abscissa) *versus* percentage inhibition (in ordinate) using Microsoft Excel. The IC_{50} value is defined as the extract concentration that inhibits the DPPH radical formation by 50%.

2.6 Evaluation of ferric reducing power
The ferric reducing power of various extracts obtained from the leaves and stem-bark of *S. dentata* was evaluated using a method outlined in the literature [12]. Briefly, a stock solution of each extract was prepared separately by dissolving 10mg of dry weight of extract in 10mL of 50% methanol (v/v in water). Further dilutions such as 5, 10, 20, 40, 60, 80 and 100µg/mL were prepared separately from each of the stock solution and followed the rest of the procedure as per literature[12]. The reducing capacity of each reaction mixture was determined by measuring its absorbance at 700nm using MRM, Mode Spectro UV-11, S/N: UEB 1704200 Spectrophotometer. The antioxidants present in the extract reduce potassium ferricyanide (Fe^{3+} salt) to potassium ferrocyanide (Fe^{2+} salt) in acidic medium. The resulting ferrocyanide ion then binds to free Fe^{3+} ions present in the ferric chloride solution to yield a Prussian blue complex[13]. The stronger the reducing power of extracts indicated by higher absorbance of the reaction mixture [13, 14].

2.7 Determination of total phenolic contents (TPCs)
TPCs of various extracts obtained from the leaves and stem-bark of *S. dentata* was determined using Folin-Ciocalteu method outlined in the literature [15]. A stock solution of tannic acid at a concentration of 1000µg/mL was prepared by dissolving 50mg of tannic acid in 50mL of 50% methanol (v/v in water). Further dilutions such as 750, 500, 250, 150, 100 and 25µg/mL were prepared from the stock solution. Extract solution at a concentration of 1000µg/mL was prepared by dissolving 10mg of each extract in 10mL of 50% methanol (v/v in water) and followed the rest of the procedure as per literature [15]. The absorbance of each mixture was measured at 760nm using MRM, Mode Spectro UV-11, S/N: UEB 1704200 Spectrophotometer. A calibration curve was obtained from regression analysis by plotting various concentrations of tannic acid (in abscissa) *versus* absorbance (in ordinate) which was used to determine TPCs of each extract. The TPCs of each extract was expressed as tannic acid equivalents (TAE) per gram of dry weight of extract.

2.8 Determination of total flavonoid contents (TFCs)
TFCs of various extracts obtained from the leaves and stem-bark of *S. dentata* was determined using the colorimetric aluminium chloride method outlined in the literature[16]. A stock solution of quercetin was prepared by dissolving in 5mg of quercetin in 5mL of 50% methanol (v/v in water). Further dilutions such as 5, 10, 15, 20, 25 and 30 µg/mL were prepared from the stock solution. Extract solution at a concentration of 1000µg/mL was prepared by dissolving 10mg of each extract in 10mL of 50% methanol (v/v in water) and followed the rest of the procedure as per literature [16]. The absorbance of each mixture was measured at 510nm using MRM, Mode Spectro UV-11, S/N: UEB 1704200 Spectrophotometer. The TFCs of each extract was determined from a quercetin calibration curve, which was obtained from regression analysis by plotting various concentrations of quercetin (in abscissa) *versus* absorbance (in ordinate). The TFCs of each extract was expressed as milligrams of

quercetin equivalent (QE) per dry weight of extract.

2.9 Statistical analysis
SPPS software version 28.0.0.0 was used to perform statistical analysis and the differences were statistically significant when $p \leq 0.05$.

3. Results
The following ten extracts were prepared from *S. dentata*. Hexane leaf extract (E1), chloroform leaf extract (E2), ethyl acetate leaf extract (E3), acetone leaf extract (E4) methanol leaf extract (E5), hexane stem-bark extract (E6), chloroform stem-bark extract (E7), ethyl acetate stem-bark extract (E8), acetone stem-bark extract (E9) and methanol stem-bark extract (E10). All these ten extracts were evaluated for DPPH radical scavenging activity and the results are summarised in Table 1. In general, all extracts showed lower scavenging activity at all concentrations compared to positive control ascorbic acid. The positive control showed scavenging activity ranging from 56.93±5.87 to 85.74±2.43% at various concentrations. The extracts from leaves showed scavenging activity ranging from7.34±1.09 to 66.28±3.29% at various concentrations. Among the leaf extracts, E3 showed highest scavenging activity of 66.28±3.29% at a concentration of 3000µg/mL. Extracts E4 and E5 also showed comparable scavenging activity as that E3 at higher concentrations. E1 showed lowest scavenging activity among leaf extracts at all concentrations. Extract E2 showed higher scavenging activity than E1 but showed lower scavenging activity than E2, E3 and E5. Similarly, the stem-bark extracts exhibited radical scavenging activity ranging from 2.67±0.86 to 77.84±8.53% at various concentrations. Extract E9 showed a scavenging activity of 9.28±0.58% at a low concentration at 200µg/mL and showed a scavenging activity of 77.84±8.53% at a high concentration at 3000µg/mL. Extract E10 showed a scavenging activity of 33.11±1.83% at 200µg/mL and showed a scavenging activity of 65.26±0.49% at 3000µg/mL. Extracts E6 and E8 showed a weak to moderate scavenging activity (Table 1). Extract E7 showed lowest scavenging activity among the stem-bark extracts at all concentrations. Extract E9 showed highest scavenging activity among stem-bark extracts at high concentration followed by E10. The percentage radical scavenging activity of these ten extracts are also given in the bar diagrams for an ease of comparison (Figure 1 and Figure 2).

Additionally, the IC_{50} values of E1-E10 were also determined using this DPPH assay. The IC_{50} values of E1-E10 and ascorbic acid are listed in Table 1. The positive control showed an IC_{50} value of <200µg/mL. All ten extracts showed relatively much higher IC_{50} values compared to positive control. In other words, all ten extracts showed relatively lower scavenging activity compared to positive control. Among the leaf extracts, E5 showed a lowest IC_{50} value of 1498.90µg/mL followed by E3 with an IC_{50} value of 1705.27µg/mL. Extracts E1, E2 and E4 showed relatively higher IC_{50} values. Among the stem-bark extracts, E10 showed a lowest IC_{50} value of 1271.29µg/mL followed by E9 with an IC_{50} value of 1673.89µg/mL. Extracts E6, E7 and E8 showed relatively higher IC_{50} values. Therefore, methanolic extracts (E5 and E10) from the leaves and stem-bark were found to be most potent among all extracts (Table 1).

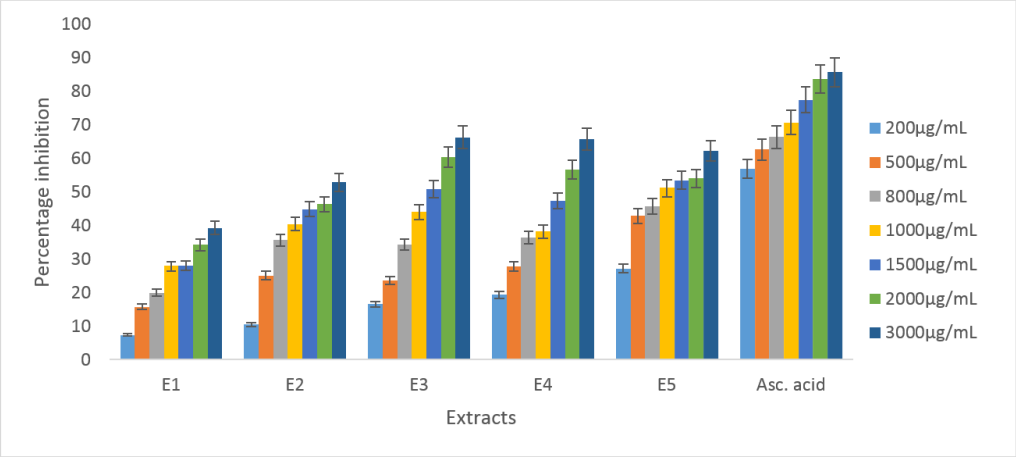

Fig 1: Percentage inhibition of DPPH radical scavenging activity of various extracts from leaves of *S. dentata*.

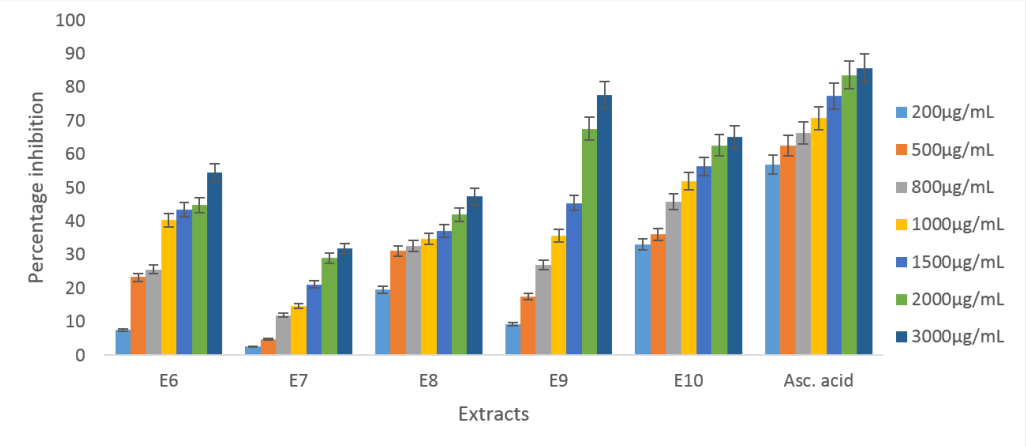

Fig 2: Percentage inhibition of DPPH radical scavenging activity of various extracts from stem-bark of *S. dentata.*

Table 1: Percentage inhibition of DPPH radical scavenging activity of various extracts from leaves and stem-bark of *S. dentata*.

Extracts	Concentrations (µg/mL)/% inhibition							IC_{50} values (µg/mL)
	200	500	800	1000	1500	2000	3000	
E1	7.34±1.09^{a}	15.73±2.09^{a}	19.94±3.21^{c}	27.90±3.65^{a}	28.07±1.97^{b}	34.24±0.63^{a}	39.72±4.37^{a}	>3000
E2	10.46±2.76^{a}	25.07±4.46^{d}	35.63±0.54^{a}	40.43±4.65^{a}	44.88±0.94^{a}	46.31±4.03^{c}	52.81±0.87^{c}	2321.16
E3	16.57±0.97^{b}	23.53±0.56^{a}	34.34±1.92^{c}	44.04±2.56^{d}	50.90±0.76^{a}	60.29±0.61^{c}	66.28±3.29^{c}	1496.27
E4	19.3±1.34^{a}	27.89±3.79^{a}	36.39±2.45^{c}	38.17±1.78^{c}	47.28±4.39^{b}	56.73±0.43^{c}	65.68±0.09^{b}	1850.58
E5	27.19±3.01^{d}	42.8±0.77^{d}	45.78±2.89^{a}	51.19±2.80^{a}	53.74±3.90^{b}	54.03±0.98^{a}	62.26±1.74^{a}	1498.90
E6	7.60±0.27^{a}	23.30±0.67^{a}	25.61±0.43^{a}	40.31±1.32^{c}	43.53±0.48^{a}	44.85±3.76^{d}	54.47±6.59^{a}	2382.55
E7	2.67±0.86^{a}	4.8±1.51^{d}	11.9±2.09^{c}	14.7±0.87^{b}	21.1±1.69^{e}	29.00±1.19^{c}	31.8±0.69^{b}	>3000
E8	19.53±2.57^{b}	31.18±1.78^{e}	32.69±0.07^{c}	34.77±0.67^{a}	37.10±2.91^{c}	41.93±3.67^{a}	47.38±1.37^{b}	>3000
E9	9.28±0.58^{a}	17.50±0.37^{a}	26.96±0.98^{e}	35.70±1.87^{b}	45.42±3.76^{d}	67.59±0.47^{b}	77.84±8.53^{b}	1673.89
E10	33.11±1.83^{e}	36.04±0.93^{a}	45.80±0.54^{b}	51.96±3.02^{a}	56.35±1.2^{b}	62.65±1.76^{a}	65.26±0.49^{c}	1271.29
Asc. acid	56.93±5.87^{b}	60.61±4.43^{a}	65.36±0.75^{a}	70.73±7.13^{a}	77.91±3.65^{a}	83.24±3.05^{a}	85.74±2.43^{a}	<200

E1 = Hexane leaf extract, E2 = chloroform leaf extract, E3 = ethyl acetate leaf extract, E4 = acetone leaf extract, E5 = methanolic leaf extract, E6 = hexane stem-bark extract, E7 = chloroform stem-bark extract, E8 = ethyl acetate stem-bark extract, E9 = acetone stem-bark extract, E10 = methanolic stem-bark extract. Asc. acid = Ascorbic acid. Values with different superscript letters are statistically different within column

Table 2: Ferric reducing power, TPCs and TFCs of various extracts from leaves and stem bark of *S. dentata*.

Extracts	Ferric reducing power at 700nm/Concentration of extracts (µg/mL)							TPCs (mg TAE/g)	TFCs (mg QE/g)
	5	10	20	40	60	80	100		
E1	0.224±0.011^{a}	0.313±0.007^{b}	0.381±0.0143^{a}	0.467±0.0466^{c}	0.568±0.0416^{a}	0.714±0.039^{b}	0.955±0.041^{c}	53.590±1.566	12.67±0.103
E2	0.069±0.005^{a}	0.087±0.004^{b}	0.102±0.0045^{a}	0.161±0.0418^{a}	0.210±0.0076^{a}	0.288±0.001^{a}	0.359±0.038^{e}	33.590±1.702	5.61±0.037
E3	0.054±0.002^{b}	0.299±0.046^{a}	0.557±0.1501^{b}	0.794±0.1843^{a}	1.049±0.0464^{c}	1.227±0.132^{c}	1.479±0.141^{b}	480.14±2.277	41.61±0.232
E4	0.342±0.048^{c}	0.591±0.056^{c}	0.745±0.0578^{d}	1.019±0.0586^{d}	1.389±0.0828^{d}	1.574±0.133^{a}	1.627±0.115^{a}	459.10±1.763^{c}	31.60±0.214
E5	0.235±0.010^{a}	0.454±0.046^{d}	0.598±0.0405^{a}	0.732±0.0487^{a}	0.888±0.0773^{c}	1.024±0.041^{d}	1.159±0.044^{c}	215.66±1.459	14.77±0.116
E6	0.237±0.031^{d}	0.322±0.004^{e}	0.389±0.0439^{c}	0.476±0.0286^{e}	0.563±0.0133^{a}	0.631±0.035^{a}	0.733±0.033^{e}	30.480±0.995	4.950±0.062
E7	0.252±0.031^{a}	0.643±0.040^{d}	0.686±0.0439^{d}	0.877±0.0285^{d}	1.118±0.0126^{b}	1.284±0.006^{b}	1.483±0.037^{b}	150.14±0.463	8.630±0.083
E8	0.373±0.009^{c}	0.461±0.481^{e}	0.692±0.2383^{a}	0.893±0.0416^{c}	1.309±0.1571^{d}	1.540±0.006^{a}	1.956±0.037^{a}	657.72±0.500	35.53±0224
E9	0.165±0.006^{a}	0.289±0.017^{a}	0.662±0.0574^{e}	0.926±±0.059^{d}	1.167±0.0437^{d}	1.312±0.104^{b}	1.568±0.009^{b}	423.24±0.458	59.08±0.371
E10	0.095±0.039^{d}	0.182±0.011^{d}	0.375±0.0595^{d}	0.704±0.0906^{a}	1.104±0.0075^{a}	1.307±0.070^{b}	1.498±0.078^{b}	371.51±0.806	29.86±0.270
Asc. acid	0.370±0.031^{d}	0.506±0.076^{a}	0.780±0.0591^{e}	0.968±0.0411^{c}	1.460±0.1270^{a}	1.901±0.036^{e}	2.041±0.047^{a}	N/A	N/A

E1-E10 = refer to footnote of Table 1. Asc. acid = ascorbic acid; TPCs = Total phenolic contents, TFCs = Total flavonoid contents; N/A = Not applicable. Values with different superscript letters are statistically different within column

The ferric reducing power of E1-E5, E6-E10 and ascorbic acid at various concentrations are summarized in Table 2. Extracts E1-E5, E6-E10 and ascorbic acid exhibited ferric reducing power in the range of 0.054±0.002 to1.627±0.115, 0.095±0.039 to 1.956±0.037 and 0.370±0.031 to 2.041±0.047 at 700nm respectively at various concentrations (Table 2). Among the leaf extracts, E4 showed highest ferric reducing power of 1.627±0.115 at 700nm at a concentration of 100µg/mL followed by E3, E5, E1 and E2 (Table 2). Extract E2 showed lowest ferric reducing power of 0.359±0.038 at 700nm at a concentration of 100µg/mL. Similarly, among the stem-bark extracts, E8 showed highest ferric reducing power of 1.956±0.037 at 700nm at a concentration of 100µg/mL followed by E7, E9, E10 and E6 (Table 2). Extract E6 showed lowest ferric reducing power of 0.733±0.033 at 700nm at a concentration of 100µg/mL.

The TPCs of E1-E10 are summarized in Table 2. The TPCs of E1-E10 was determined using tannic acid calibration curve, which is shown in Figure 3. The TPCs of E1-E5 and E6-E10 were determined to be in the range of 33.590±1.702 to 480.14±2.277 and 30.480±0.995 to 657.72±0.500mg TAE/g of dry weight of extract, respectively. Extracts E3 and E8 showed highest TPCs among leaf and stem-bark extracts, respectively (Table 2).

The TFCs of E1-E10 are summarized in Table 2. The TFCs of E1-E10 was determined using quercetin calibration curve, which is shown in Figure 4. The TFCs of E1-E5 and E1-E5 were determined to be in the range of 5.61±0.037 to 41.61±0.232 and 8.630±0.083 to 59.08±0.371mg QE/g of dry weight of extract, respectively. Extracts E3 and E9 showed highest TFCs among leaf and stem-bark extracts, respectively (Table 2).

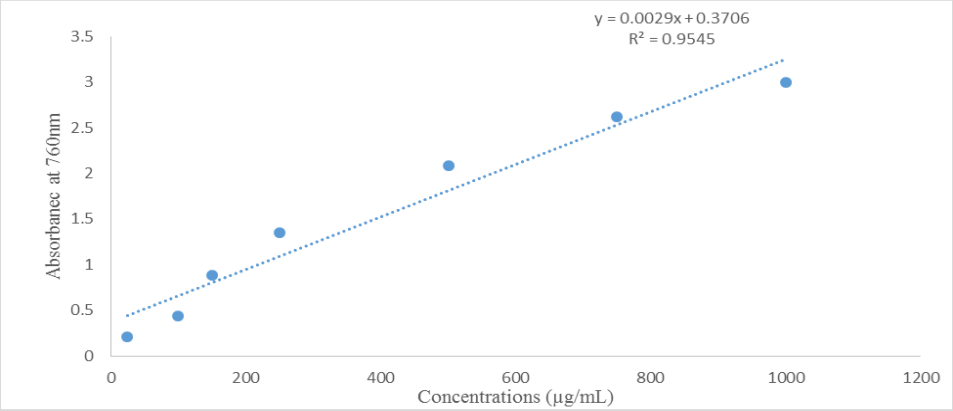

Fig 3: The calibration curve of tannic acid, which is used to estimate the TPCs of various extracts from *S. dentata*.

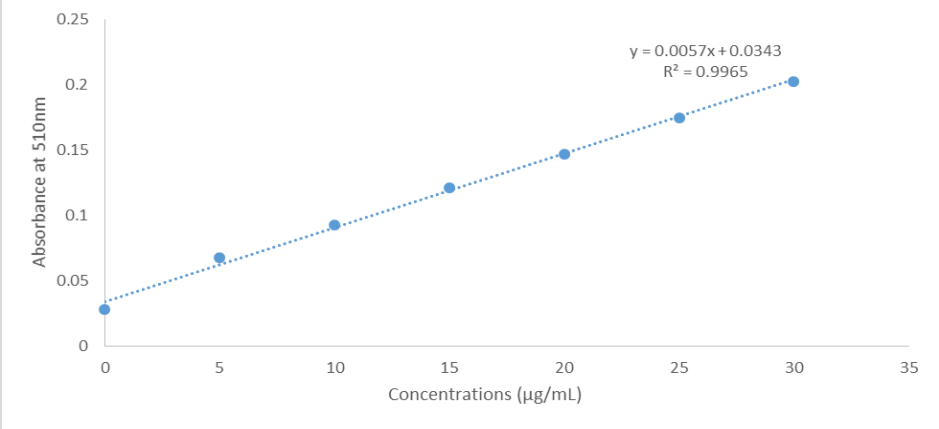

Fig 4: The calibration curve of quercetin, which is used to estimate the TFCs of various extracts from *S. dentata*.

4. Discussion

Various species from the Searsia genus such as *S. dentata*, *S. chirindensis*, *S. natalensis* and *S. pyroides* have been used in the treatment of epilepsy and convulsions in South Africa [17-19]. The ethanolic extract from leaves of *S. dentata* has been exhibited activity against sedentary death syndrome (SED) and the ED_{50} value was found to be 0.62mg dry extract/mL [19]. The ethanolic extract from leaves has also been showed activity against epilepsy and binding activity to flumazenil-sensitive site on the GABAA receptor [20]. Flavonoids such as gathisflavone, amentoflavone and apigenin have been isolated from this ethanolic leaf extract and identified as active compounds for activity against epilepsy [20]. However, these three flavonoids have been exhibited no effects on the SED at a concentration of 0.01-100 M [19]. Phenolics and flavonoids have hydroxyl groups in the aromatic rings and donate the acidic hydrogens readily to scavenge harmful free radicals [21, 22]. Our study showed that various extracts from leaves and stem-bark of *S. dentata* have phenolics and flavonoids at various proportions and their presence justified the radical scavenging activity in this study.

5. Conclusions

Hexane, chloroform, ethyl acetate, acetone and methanol extracts were obtained separately from leaves and stem-bark of *S. dentata*. These extracts were evaluated for DPPH radical scavenging activity and ferric reducing power. The IC_{50} values of these extracts were also determined by DPPH assay. Additionally, the TPCs and TFCs of these extracts were determined. In general, all extracts showed relatively lower scavenging activity compared to positive control ascorbic acid but showed significant scavenging activity. Acetone stem-bark extract showed a highest scavenging activity among all extracts. The methanolic stem-bark extract showed a lowest IC_{50} value among all extracts. The ferric reducing power of these extracts also found to be relatively lower than positive control ascorbic acid. The ethyl acetate stem-bark extract showed a highest ferric reducing power among all extracts and its ferric reducing power was very much comparable to positive control. The chloroform leaf extract showed a lowest ferric reducing power among all extracts. The ethyl acetate stem-bark extract and hexane leaf extract showed highest and lowest TPCs respectively among all extracts. On the other hand, the acetone stem-bark extract and hexane stem-bark extract showed highest and lowest TPCs respectively among all extracts. From this study, we concluded that *S. dentata* could be a valuable source of antioxidants. Further studies on this plant are required to explore its efficacy in the therapeutic applications.

6. Conflict of interest statement

The authors declared that there is no conflict of interests.

7. Acknowledgements

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