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Full Length Research Paper

Antioxidant and free radical scavenging properties of four plant species used in traditional medicine in Lesotho

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Among the top ten causes of death in Lesotho are degenerative diseases such as heart failure, anaemia, *diabetes mellitus* and stroke, which are generally linked to oxidative stress. The therapeutic benefit of medicinal plants in the treatment of degenerative diseases is attributed to their antioxidant properties. In the present study, crude extracts of four plant species used in ethnomedicine in Lesotho were evaluated for their antioxidant activities using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging, reducing power, hydrogen peroxide scavenging and total phenolics. The species were *Leucosidea sericea* (leaves), *Berkheya setifera* (corm), *Trifolium burchellianum* (leaves, stems and roots) and *Polygala virgata* (leaves and stems). *Camellia sinensis* (green tea) was included as a standard as it is known to have antioxidant activity. Results showed that these plants scavenged DPPH radicals and hydrogen peroxide, reduced Fe^{3+} to Fe^{2+} and contained phenolics. A fair correlation between total phenolics and antioxidant activity was observed. *L. sericea* and *B. setifera* with the highest content of phenolics (149.63 and 66.0 mg GAE/g dry extract respectively) were also more potent in all the antioxidant assays. The results suggested that phenolics in these plants could be partly responsible for their beneficial therapeutic effects.

Keywords: Ethnomedicine, phenolics, plant extracts, DPPH, GAE.

INTRODUCTION

In most industrialized countries, cardiovascular disease and cancer are ranked as the top two leading causes of death and the causes of both diseases have been linked to lifestyle choices, particularly diet (Boyer and Liu, 2004; Patel et al., 2010; Ndhlala et al., 2013). In the Kingdom of Lesotho, data obtained from the World Health Organisation and from the Ministry of Health and Social Welfare showed that four degenerative diseases namely, heart failure, anaemia, *diabetes mellitus* and stroke, are among the top ten causes of death in government hospitals. Together, the four diseases accounted for about 7% of all deaths in 2005 and 13.89% of all deaths in 2010 (WHO, 2010).

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as by-products of biological reactions or from exogenous factors (Ames, 1998). *In vivo*, some of these ROS play positive roles in cell physiology (Ames *et* al., 1993); however, they may also cause great damage to cell membranes (Pryor, 1973), induce oxidation that causes membrane lipid peroxidation and decreased membrane fluidity (Pryor, 1973), mutations in DNA leading to cancer (Cerutti, 1991; Ames et al., 1993; Chahar et al., 2011) and other degenerative diseases (Harman 1994; Finkel and Holbrook, 2000; Wong et al., 2012).

However, in one study, it was found that people who ate the highest amount of fruits and vegetables had a 20% lower risk of coronary heart disease; the lowest risks were observed in people who consumed more green leafy vegetables and fruits rich in vitamin C (Joshipura *et. al.* 2001). It has also been suggested that a diet high in fruits and vegetables may help protect against cataracts, diabetes, Alzheimer disease (Willett, 2002, Ames, 1993) and even asthma (Woods et. al., 2003). Much of the protective effect of fruits and vegetables has been attributed to phytochemicals, which are the non-nutrient plant compounds such as the carotenoids, flavonoids,

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isoflavonoids and phenolic acids. A major role of these phytochemicals is protection against oxidation (Boyer and Liu, 2004; Wong et al., 2012). These compounds are thought to act as a proton sink that synergistically bring about the antioxidant and free radical scavenging potentials observed (Alisi et al., 2011; Ndhlala et al., 2013).

The multiple hydroxyl groups which are bonded directly to the aromatic ring of phytochemicals allow them to act as free radical scavengers, reducing agents, hydrogen donors and singlet oxygen quenchers (Hatano et al., 1989; Barnerjee et al., 2008; Uyoh et al., 2013; Ndhlala et al., 2013). Phytochemicals donate electrons easily to electron-seeking free radicals, thereby reducing damage in living cells (Uyoh et al., 2013). Phenolic compounds are powerful chain breaking primary antioxidants associated with cellular antioxidant activity and play a crucial role in stabilizing lipid peroxidation (Shahidi and Wanasudara, 1992; Yen et al., 1993; Muchuweti et al., 2006). Antioxidants, such as vitamin A, C, and beta carotene, can neutralize the ill effects of free radicals by scavenging or chain breaking or some other mechanism of action and must therefore be constantly replenished since they are 'used up' in the process of neutralizing free radicals (Kumar and Sharma, 2006). It has also been suggested that phenolic compounds inhibit auto-oxidation of unsaturated lipids, thus preventing the formation of oxidized low density lipoprotein (LDL), which causes cardiovascular diseases (Alisi et al., 2011). Phytochemicals are thought to inhibit cancer cell proliferation (Ames et al., 1993; Chahar et al., 2011), regulate inflammatory and immune response (Hollman and Katan, 1997) and protect against lipid oxidation (Liu, 2003; Patel et al., 2010). A direct relationship between antioxidant activity and total phenolic content of plant extracts has been reported (Larson, 1988; Kaur and Kapoor, 2002; Sharma and Joshi, 2011; Katerere et al., 2012). The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (Hertog et al., 1993; Zhang et al., 2001). Thousands of phytochemicals have been identified in foods, yet there are still many that have not been identified. Therefore the quest for antioxidants of plant origin with broad-spectrum actions has increased during the last three decades, as evidenced by the increase in the production of antioxidant-based drugs and formulations for the prevention and treatment of complex diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam, 2004; Akinpelu et al., 2010).

In the present study, the crude methanolic extracts of four plant species used in traditional medicine in Lesotho were evaluated for their antioxidant activities and content of total phenolics using the following assays: DPPH free radical scavenging, reducing power with potassium ferricyanide, hydrogen peroxide scavenging and content of total phenolics using the Folin-Ciocalteu phenolic reagent. The evaluated plant species were *Leucosidea sericea* (Rosaceae), *Berkheya setifera* (Asteraceae), *Trifolium burchellianum* (Fabaceae) and *Polygala virgata* (Polygalaceae). Medicinal plants, as discussed at the 1st NEPAD/SANBio workshop on scientific validation of traditional medicine for affordable treatment of HIV / AIDS and opportunistic infections, are used in traditional medicine in Lesotho (Saka, 2007) and considered as the most appropriate intervention in the health care needs in Lesotho (Saka, 2007) to cure or suppress diseases such as cancer, arthritis and cardiovascular diseases (Maliehe, 2007). *Camellia sinensis* (Theaceae) was included as a standard for comparison because it is known to have antioxidant activity.

Leucosidea sericea, known as cheche in Sesotho**,** is used as a tonic. The fresh green leaves are harvested, boiled in water and the concoction can then be taken (Van Wyk and Van Wyk, 1997). The active compounds of this plant were found to be the phloroglucinol derivatives; aspidinol and desaspidinol, which were previously reported to be present in a *Dryopteris* species (Bosman *et al.,* 2004).

Berkheya setifera is known as leleme la khomo in Sesotho (Van Wyk and Van Wyk, 1997). The corm of this plant is used as a concoction for removal of toxins from the blood and as a tonic by Lesotho traditional healers (Van Wyk and Van Wyk, 1997).

Trifolium burchellianum is known as musa-pelo/morokho in Sesotho (Van Wyk and Van Wyk, 1997). In Lesotho, the Burchell's clover is used as a sedative for those with high blood pressure, used for cleaning impurities in the blood and as an antidote for cancer. *T. burchellianum* contains no prussic acid and has a low level of fraction 1 or 18S protein, a protein, which in high levels is often associated with stable foam in bloat (Wilson and Bowman, 1993). The species is therefore considered non-toxic.

Polygala virgata is indigenous to Lesotho and known as nts'ebele ea moru in Sesotho (Van Wyk and Van Wyk, 1997). In Lesotho, the leaves and stems are prepared and used as blood purifiers (Van Wyk and Van Wyk, 1997). The active ingredients of *Polygala* species include flavonols (especially kaempferol or quercetin or kaempferol and quercetin) and saponins can also be present (Watson and Dallwitz, 1992).

Camellia sinensis is a plant that is native to mainland South and Southeast Asia, especially India and China (Ming, 1992). The leaves are used in the production of green tea. Green tea (non-fermented) is derived directly from drying and steaming of fresh tea leaves (Zuo *et al.,* 2002). Green tea contains mainly polyphenolic catechins such as (-)-epicatechin, (+)-gallocatechin, (+)-catechin as well as gallic acid and caffeine, to which some of the beneficial effects of tea have been attributed (Gupta *et al.,* 2002). *Camellia sinensis* was included in the study so that it could be used for comparative analysis since its phytochemistry, antioxidant activity and biochemistry have been extensively studied.

MATERIALS AND METHODS

MATERIALS

Chemicals

All chemicals and solvents were of analytical grade, obtained from suppliers in the Republic of South Africa. Ethanol, potassium ferricyanide, ferric chloride, sodium carbonate, potassium iodide, sulphuric acid, hydrochloric acid and L-Ascorbic acid were products of Saarchem-Holpro Analytical (Republic of South Africa); Folin-Ciocalteau reagent, phenol, gallic acid, catechin hydrate and 1, 1-Diphenyl-2- picrylhydrazyl (DPPH) were products of Sigma-Aldrich (Germany); linoleic acid Sigma-Aldrich (India); ferrous chloride, trichloroacetic acid, ammonium molybdate, hydrogen peroxide, ammonium thiocyanate, sodium carbonate, sodium thiosulphate, aluminium chloride, sodium nitrite and methanol were products of Fisher Scientific (UK); Tris-HCl (Merck Chemicals, Germany); sodium hydroxide and phosphate buffer (Rochelle Chemicals, Republic of South Africa).

Apparatus

UV-1201 Spectrophotometer (SHIMADZU), Kyoto, Japan.

Plant material and crude plant extracts

The plant parts used were as follows: *Leucosidea sericea* and *Camellia sinensis (*leaves); *Trifolium burchellianum* (leaves, stem and roots); *Polygala virgata* (leaves and stem) and *Berkheya setifera* (corm). The plants were collected from the outskirts of the National University of Lesotho, and authenticated by the curator of the Herbarium at the Department of Biology of university, where the voucher specimens of these plants have been deposited.

METHODS

Preparation of crude extracts

The crude extracts were prepared according to the method of Adedapo *et al* (2009), with slight modifications. The plant materials were dried under shade till leaves were brittle and ground into powder using a porcelain pestle and mortar. The ground plant materials of *P. virgata*, *T. burchellianum* and *C. sinensis* were soaked in 98% methanol with shaking on an orbital shaker for 7 days. The ground plant materials of *L. sericea* and *B. setifera* were soaked in methanol: water (9:1) solution and shaken on an orbital shaker for 24 hours. The extracts were filtered through No. 1 Whatman filter paper (Whatman Ltd., England) using suction. The filtrates obtained were concentrated under vacuum in a rotary evaporator and oven dried at 35ºC overnight to constant weight. The extracts were stored at 4ºC until used.

Determination of DPPH free radical scavenging activity

The free radical scavenging activity of the extract was evaluated for hydrogen ion donating or free radical scavenging ability using the stable free radical DPPH method of Blois (1958) with some modifications. Plant extract solutions were prepared by dissolving 0.03g powder in 10mL of 50% methanol and making serial dilutions to obtain different concentrations, in μgmL^{-1} (3 000, 2 500, 2 250, 2 000, 1 750, 1 500, 1 250, 1 000, 500 and 200). A 0.1mM solution of DPPH in methanol was also prepared. Each reaction mixture contained a 0.05mL sample of plant extract solution, 1.0mL of 0.1mM DPPH solution and 0.45 mL of 50mM Tris-HCl buffer (pH 7.40). The contents were mixed and then incubated at room temperature for 30minutes. DPPH radicals react with suitable reducing agents, such as plant extracts, and are reduced to the corresponding hydrazine as the electrons become paired off. The solution changes colour stoichiometrically from deep violet to yellowish, depending on the number of electrons taken up. The absorbance of the mixture was then measured at 517nm against the corresponding blank solution (50% v/v methanol in distilled water). The assay was performed in triplicate. L-Ascorbic acid was used as positive control (Saeed *et al.,* 2012) and (+)-catechin was used for comparative analysis.

Percentage inhibition of DPPH free radical was calculated based on the control reading, which contained DPPH and distilled water without any extract using the following equation:

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

where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of extract was expressed as IC_{50} (Ndhlala *et al.,* 2013). The IC_{50} value was defined as the concentration (in µg/mL) of extract that inhibited the formation of DPPH radicals by 50% (Ho *et al* 2012; Moyo *et al.,* 2013).

Reducing power assay

The reducing power assay was carried out according to the method of Yildirim *et al* (2001). The transformation of the yellow colour of the Fe^{3+}/ferricyanide complex to Perl's Prussian blue colour of the ferrous (Fe^{2+}) form was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power

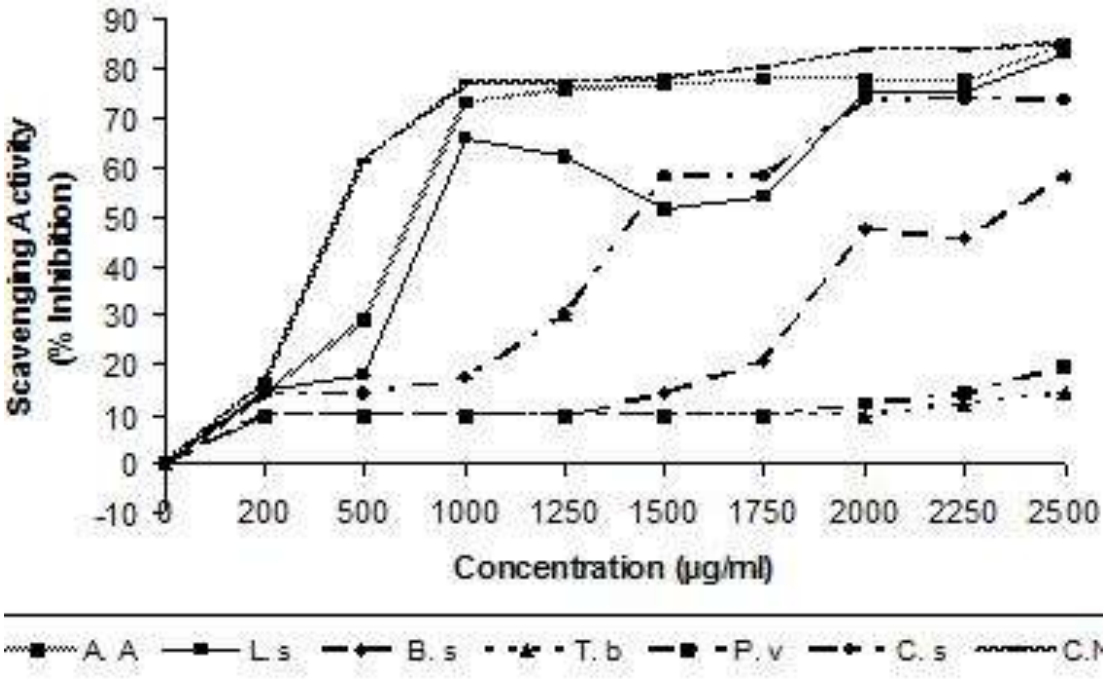

Figure 1. DPPH free radical scavenging activity of different concentrations of the plant extracts plus ascorbic acid and catechin standards. Each value represents the means ± s.d. (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*; A.A = Ascorbic acid; C.N = Catechin.

(Evanjelene and Natarajan, 2011).

A 100µL aliquot portion of 20, 40, 60, 80 or 100µgmL^{-1} of plant extract dissolved in 50% methanol, was mixed with 2.5mL phosphate buffer (0.2M, pH 6.60) and 2.5mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The mixture was incubated at 50ºC for 30 minutes. The reaction was stopped by adding trichloroacetic acid (2.5mL, 10%) to the mixture, which was then centrifuged at 3 000 rpm for 10 minutes. Then 2.5mL of the upper layer of the supernatant was mixed with 2.5mL distilled water and 0.5mL freshly prepared $FeCl_3$ (0.1%) and the absorbance was measured at 700nm. The assay was performed in triplicates. Ascorbic acid solutions (0 - 1 000µgmL^{-1}) were used as reference. Reducing power was given in ascorbic acid equivalents (AAE) in milligram per gram of dry extract.

Determination of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of plant extracts was estimated by replacement titration according to the method of Zhang (2000), with slight modifications. The assay was performed by mixing 1.0mL of hydrogen peroxide solution (0.1mM) and 100µL of various concentrations of extracts, followed by 2 drops of 3% ammonium molybdate, 10mM solution of sulphuric acid (2.0M) and 7mL of potassium iodide (1.8M). The mixed solution was titrated with 5.09mM solution of sodium thiosulphate until the yellow color disappeared. The percentage of scavenging of hydrogen peroxide was calculated thus:

Hydrogen peroxide scavenged (%) = $[(V_{cont} - V_{test})/V_{cont}] \times 100$

where, V_{cont} was volume of sodium thiosulphate used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_{test} was the volume of sodium thiosulphate solution used in the presence of extract.

Determination of total phenolic content in the crude extracts

Determinations of the total phenolic content of the extracts were done by the method of McDonald *et al* (2001), with slight modifications (Sharma and Joshi, 2011). A 1.0mL volume of plant extract solution (5.0gL^{-1} in ethanol) was mixed thoroughly with Folin-Ciocalteu reagent (1.0mL) in a volumetric flask. After 3 minutes, 4.0mL of sodium carbonate (Na_2CO_3) solution (0.70M) was added, then the mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer.

A standard calibration curve was obtained by mixing 1mL ethanolic solutions of gallic acid of various concentrations (0.025–0.400mgmL^{-1}) also with 1.0mL Folin-Ciocalteau reagent (ten-fold dilution) and 4.0mL sodium carbonate (0.7M). The absorbance of the solutions was measured at 760nm. All determinations were carried out in triplicate. The total phenolic content of each extract was determined in milligrams of gallic acid equivalents (GAE)

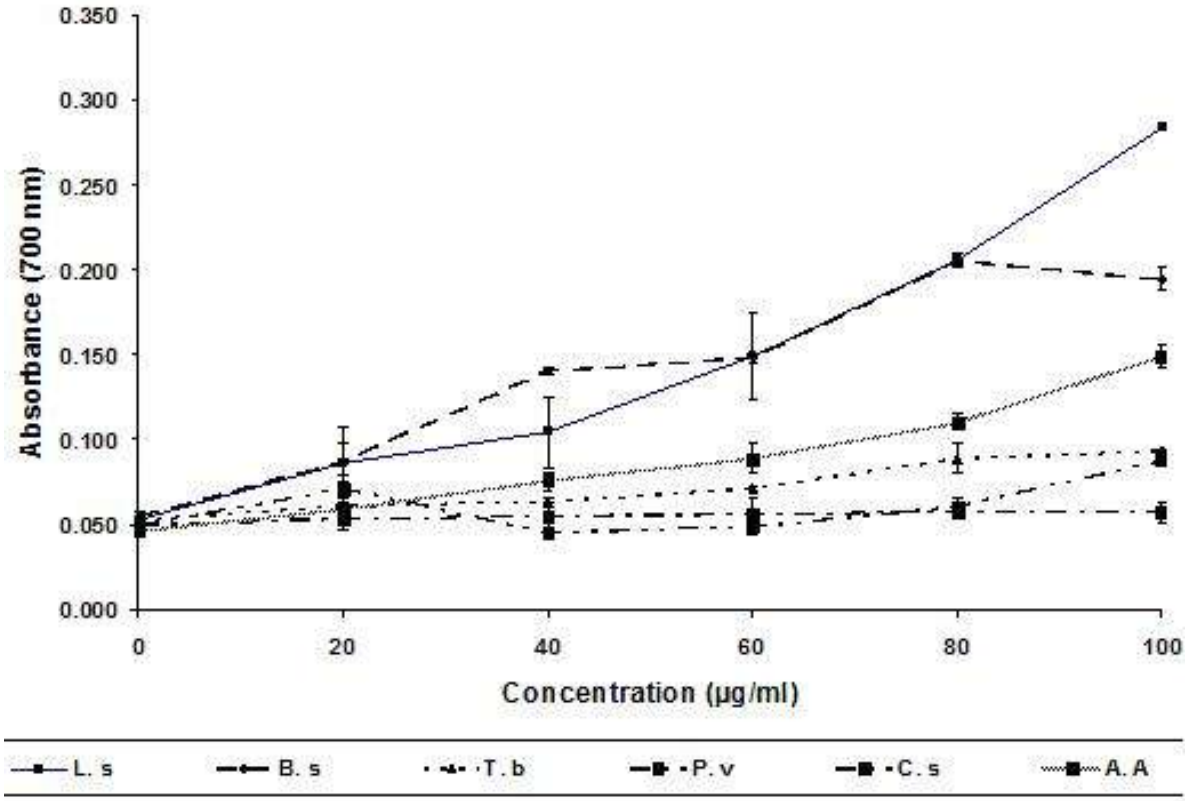

Figure 2. Reducing power of different concentrations of the plant extracts. Each value represents the mean ± s.d. (n = 3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*; A.A = Ascorbic acid.

per gram dry extract, from the gallic acid standard curve (Wong *et al.,* 2012; Moyo *et al.,* 2013).

Statistical analysis

Tests were carried out in triplicate. The amount of extract needed to inhibit free radical concentration by 50%, IC_{50}, was graphically determined from the trend line of the curve of percent (%) inhibition (y-axis) against concentration (μgmL^{-1}) (x-axis). Where the straight line from the 50% inhibition on the y-axis intersected the trend line, gave the CI_{50} (μgmL^{-1}) on the x-axis. The optical density (o.d) readings for each concentration was expressed as the mean (□) ± standard deviation (s.d).

RESULTS

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

When reduced in the presence of the antioxidants DPPH solution changes from purple to yellow resulting in a decrease in absorbance of the test solutions. In Figure 1 is illustrated the decrease in the concentration of DPPH radicals due to the scavenging ability of the five plant extracts and the ascorbic acid and the catechin standards used in this study. A concentration of 2 500μgmL^{-1} of ascorbic acid, *L. sericea*, *B. setifera*, *T. burchellianum*, *P. virgata*, *C. sinensis* and catechin exhibited 86, 83, 58, 14, 20, 74 and 86% inhibition of DPPH free radical activity respectively. The IC_{50} (µg/mL) values for ascorbic acid, *L. sericea, B. setifera, C. sinensis* and catechin were found to be 750, 850, 2 335, 1 440 and 425 respectively. A concentration of 2 500μgmL^{-1} of *T. burchellianum* and of *P. virgata* exhibited 14% and 20% inhibition of DPPH activity respectively.

Reducing power of crude plant extracts

The reducing power of the mixtures increased with increasing concentration of the plant extracts in the $K_3[Fe(CN)_6]$ reaction mixture, as shown by the increase in absorbance at 700nm within a concentration range of 10 – 100μgmL^{-1} (Figure 2). The reducing power potency was

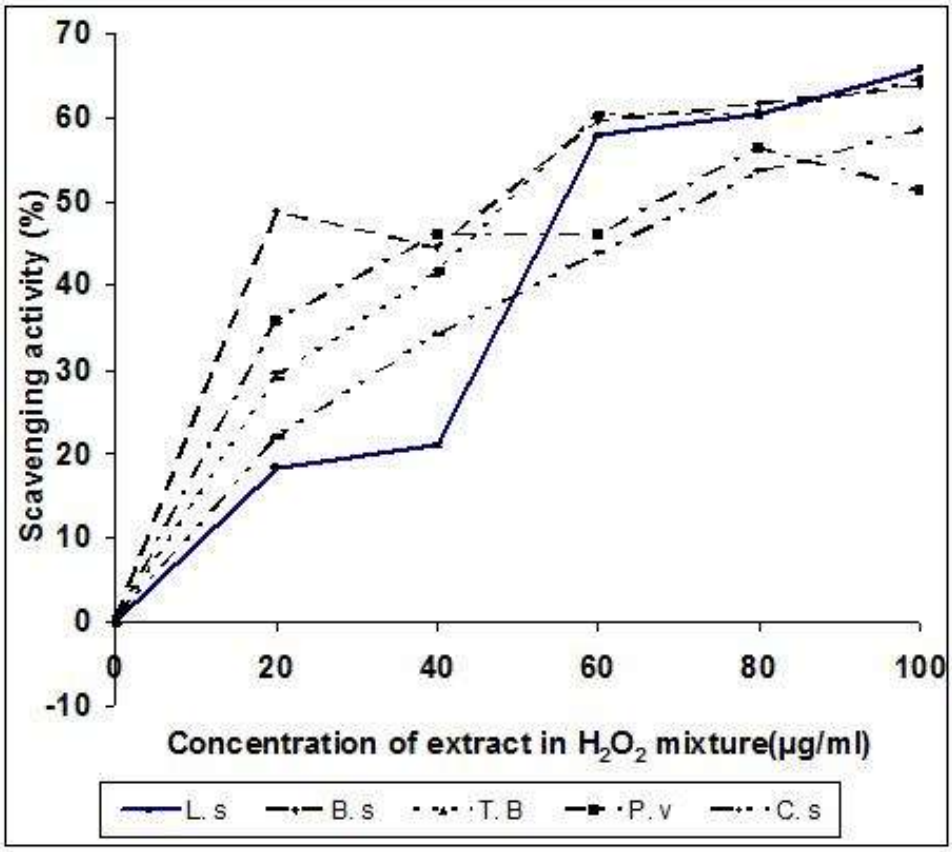

Figure 3. H_2O_2 radical scavenging activity of the five plant extracts at different concentrations. Each value represents the mean ± s.d. (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*.

in the order; *L. sericea* > *B. setifera* > ascorbic acid > *T. burchellianum* > *C. sinensis* > *P. virgata*.

Hydrogen Peroxide Scavenging Activity

The extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner (Figure 3).

At 100µgmL^{-1} extract in the reaction mixture, the % inhibition and IC_{50} values (in µgmL^{-1}) of the extracts were: *L. sericea* (66 and 68); *B. setifera* (65 and 55); T. *burchellianum* (65 and 62); *P. virgata* (51 and 74) and *C. sinensis* (59 and 75) respectively.

Total Phenolics in crude extracts

The total phenolic content of the plant extracts was determined in mg of gallic acid equivalent per gram of dry extract (mgGAE/g dry extract) using the equation obtained from the standard gallic acid graph. The equation was: y = 0.0723x - 0.0358. The amount of total phenolics obtained as mgGAE/g dry extract was found to vary considerably between plant extracts as shown in Figure 4. With regard to the total content of phenolics, in mgGAE/g dry extract, the plant extracts ranked as follows: *C. sinensis* (183.19) > *L. sericea* (149.63) >*B. setifera* (66.0) > *P. virgata* (59.91) > *T. burchellianum* (21.92).

Figure 1: DPPH free radical scavenging activity of different concentrations of the plant extracts plus ascorbic acid and catechin standards. Each value represents the means ± s.d. (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*; A.A = Ascorbic acid; C.N = Catechin.

Figure 2: Reducing power of different concentrations of the plant extracts. Each value represents the mean ± s.d. (n = 3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*; A.A = Ascorbic acid.

Figure 3: H_2O_2 radical scavenging activity of the five plant extracts at different concentrations. Each value represents the mean ± s.d. (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*.

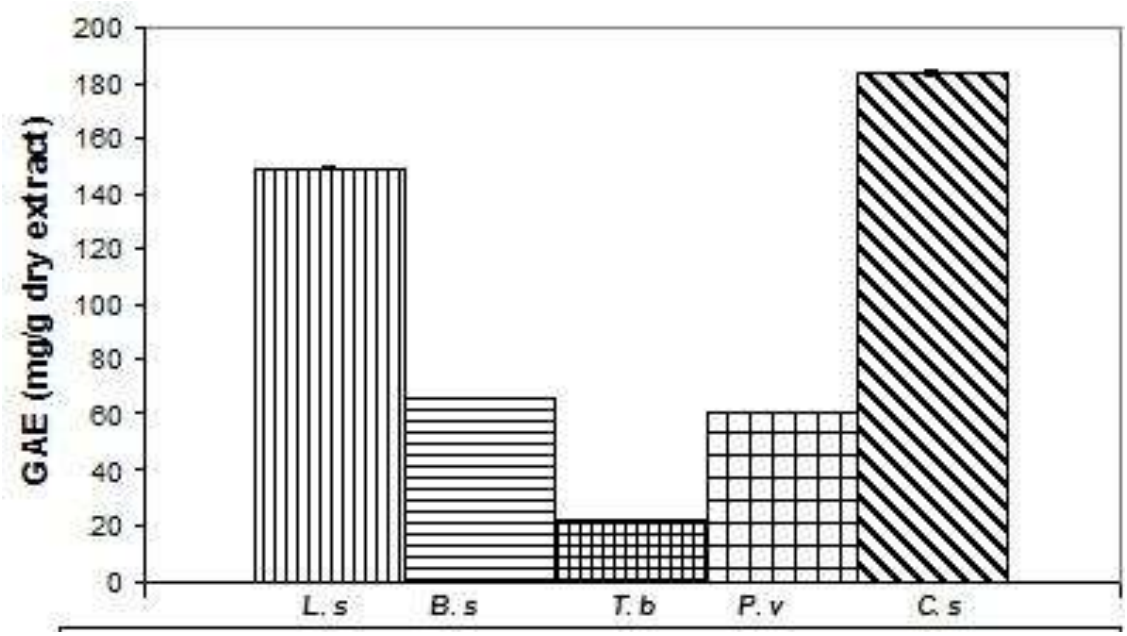

Figure 4. Total content of phenolics in plant extracts in Gallic Acid Equivalents [GAE (mg/g of dry plant extract)]. Each value represents the mean ± SD (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*.

Figure 4: Total content of phenolics in plant extracts in Gallic Acid Equivalents [GAE (mg/g of dry plant extract)]. Each value represents the mean ± SD (n=3). *L.s = L. sericea*; *B.s = B. setifera*; *T.b = T. burchellianum*; *P.v = P. virgata*; *C. s = C. sinensis*.

DISCUSSION

Free radicals have been implicated in many disease conditions, and herbal drugs containing free radical scavengers are gaining importance for the treatment of such diseases (Patel *et al.,* 2010). The decrease in absorbance of DPPH radical, caused by the reaction between antioxidant molecules in the extracts and DPPH free radical, results in the scavenging of the DPPH free radicals by hydrogen donation. It was visually noticeable as a change in color from deep violet to yellow. The free radical DPPH is commonly used as a substrate to evaluate primary antioxidative activity (Chang *et al.,* 2002).

In the present study, different methanolic plant extracts reduced DPPH free radicals in a concentration dependent manner which was in agreement with the results of Guneshwor and Haripyaree (2012). The values of percent inhibition of DPPH free radicals are shown in Figure 1. The reductions in the concentration of DPPH free radicals due to scavenging by the different extracts, ascorbic acid and catechin standards are also shown in Figure 1. The mechanism of reaction between antioxidants and DPPH is dependent upon the structural conformation of the antioxidants which could be attributed to their hydrogen donating ability (Sunmonu and Afolayan, 2012). At the concentration of 2 500µgmL^{-1} only the extracts of *T. burchellianum* and of *P. virgata* failed to inhibit 50% of DPPH free radical activity. They only inhibited 14% and 20% of DPPH free radical activity respectively.

The results indicated that the plant extracts contained antioxidants which reduced the DPPH free radical to the corresponding hydrazine (Sanchez-Moreno, 2002). In terms of potency, the mixtures ranked as follows: Ascorbic acid = catechin > *L. sericea* > *C. sinensis* > *B. setifera* > *P. virgata* > *T. burchellianum*. None of the plant extracts showed greater inhibitory activity than the ascorbic or the catechin standards used. However, *L. sericea* showed greater DPPH free radical scavenging than *C. sinensis*. Our findings on the antioxidant activity of the medicinal plants used in this study thus corroborated the results of similar studies by other authors (Saleem et al., 2001; Lai et al., 2001; Jayaprakash et al., 2001; Aqil et al., 2006; Chanda et al*.,* 2011).

For the measurement of the reducing power of the plant extracts, we investigated the transformation of Fe^{3+} to Fe^{2+} ion in the presence of the plant extracts, which served as an indicator of their antioxidant activity (Meir *et* al., 1995; Olorunnisola et al., 2012). The curves of the reducing power of the plant extracts are presented in Figure 2. The reducing power of the extracts increased with increasing concentrations as shown by the increase in absorbance at 700nm within a concentration range of 10-100µgmL^{-1}. At 100µgmL^{-1}, the potency was in the order; *L. sericea* > *B. setifera* > ascorbic acid > *T.*

burchellianum > *C. sinensis* > *P. virgata*. All extracts exhibited dose-dependent antioxidant activities, also observed by Uyoh *et al* (2013) in a different study. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain reaction initiation through donation of electrons, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and free radical scavenging (Diplock, 1997; Alisi et al., 2011). Compounds possessing reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they act as primary and secondary anti-oxidants (Sharma and Joshi, 2011).

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. According to Miller *et al* (1993), hydrogen peroxide can cross cell membrane rapidly and, once inside the cell, hydrogen peroxide can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. It is therefore biologically beneficial for cells to control the accumulated hydrogen peroxide. Figure 3 clearly shows that the extracts demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner. The order of decreasing potency of the IC_{50} values (μgmL^{-1}) : *B. setifera* (55) > T. *burchellianum* (62) > *L. sericea* (68) > *P. virgata* (74) > *C. sinensis* (75).

The total phenolic content of the plant extracts was determined by the Folin- Ciocalteu phenolic reagent method of McDonald *et al* (2001). This assay detects phenolic acids, flavonoids, tannins, anthocyanins, lignans and coumarins (Katerere *et al.,* 2012).

The extracts of the four medicinal plants contained varying amounts of phenolics and, in the content of total phenolics, expressed as mgGAE/g dry extract, ranked as follows: *L. sericea* (149.63) >*B. setifera* (66.0) > *P. virgata* (59.91) > *T. burchellianum* (21.92). The ranking of the plants according to the content of total phenolics was similar to the ranking on the basis of their DPPH free radical scavenging activities which was: *L. sericea* (83%) > *B. setifera* (58%)> *P. virgata* (20%) > *T. burchellianum* (14%). The ranking of the extracts on the basis of their DPPH free radical scavenging potency was identical to ranking them on the basis of their reducing power and hydrogen peroxide scavenging. Thus a fair correlation between total phenolic content and antioxidant activity was observed. A similar observation was made in a similar study with plants by Akbari *et al* (2012) who also observed a strong correlation between total phenolic content and free radical scavenging activity that was however, the correlation was not replicated in studies with different parts of a walnut fruit. The variation in antioxidant activities between extracts of different plant species or parts of the same plant has been attributed to differences in the population of phenolic compounds in different plants/parts of a plant. Such differences in the content and activities of phytochemicals, it has been suggested, are controlled at the level of the gene (Akbari *et al.,* 2012; Katerere *et al.,* 2012).

Among the four plants used for traditional medicine, *L. sericea*, with the highest content of phenolics was also most potent at DPPH free radical scavenging, and had the highest reducing power of Fe^{3+} to Fe^{2+} ion. This positive correlation between total phenolic content and DPPH free radical scavenging has been observed by other researchers (Chanda *et al.,* 2011). The total phenolic content of *C. sinensis* of 183.19mg/g determined in the present study compared favorably with the value of 163.33mg/g that was determined by Aqil *et al* (2006). Our results suggested that the plants used in traditional medicine in Lesotho which were evaluated in the present study contained varying concentrations of total phenolics and exhibited antioxidant activities, but to different degrees, in the different assays. These findings are in agreement with similar studies with plants and support the view that some plants used in traditional medicine can be sources of antioxidants which can be used for the treatment and management of diseases caused by free radicals (Lipinski, 2011; Olorunnisola *et al.,* 2012).

CONCLUSIONS

The results of the present study showed that the methanolic extracts of parts of four plants used in traditional medicine in Lesotho namely, *L. sericea*, *B. setifera*, *T. burchellianum* and *P. virgata* scavenged DPPH free radicals, reduced Fe^{3+} to Fe^{2+}, scavenged hydrogen peroxide and contained phenolics. A fair correlation between total phenolics content and antioxidant activity was observed. *L. sericea and B. setifera* with the highest content of phenolics (149.63 and 66.0 mg GAE/g dry extract respectively) were also more potent in all the antioxidant assays used in this study.

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