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In vitro antioxidant, anti-lipid peroxidation activities and HPLC analysis of methanol extracts from bark and stem of *Mahonia leschenaultia* takeda

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ABSTRACT

The antioxidant activity, anti-lipid peroxidation activity, polyphenolic contents and HPLC analysis of bark and stem of Mahonia leschenaultia takeda was investigated. Total phenolic and flavonoid contents were determined using folin-ciocalteu reagent method and aluminium chloride method respectively. Antioxidant activity was evaluated by the following methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) quenching assay, reducing capacity, scavenging capacities towards hydroxyl ion radicals ($^{\cdot}OH$), nitric oxide (NO), Hydrogen peroxide (H_2O_2); iron chelating capacity, ferric reducing antioxidant power (FRAP) and anti-lipid peroxidation assays. The bark extract exhibited 5346.1 ± 1.040 mg GAE/100g phenolic content, 4317.2 ± 0.946 mg QE/100g flavonoid content and 5346.3 ± 0.720 mg QE/100g flavonol content. The stem extract afforded a phenolic content of 5255.8 ± 0.644 mg GAE/100 g, flavonoid content of 3082 ± 0.917 mg QE/100 g and flavonol content of 3363.3 ± 0.544 mg GAE/100 g. The methanolic extracts from bark (EC_{50} 40 ± 1.34) and stem (EC_{50} 50 ± 1.10) exhibited high scavenging capacity towards DPPH, nitric oxide and hydrogen peroxide. The reductive capacity and FRAP of the extracts increased with increasing concentration of the samples. The antioxidant capacities of the extracts were comparable with the antioxidant standards, butyl hydroxy toluene (BHT) and EDTA. Significant and positive correlations were observed between polyphenolic contents and antioxidant capacities, indicating that the phenolics were major contributors of the antioxidant property. The HPLC analysis of the extracts showed the presence of the alkaloid Berberine. The results of the present study revealed that the bark and stem of M. leschenaultia are the natural radical scavengers with potent antioxidant activity.

Keywords: *M. leschenaultia*, antioxidant activity, FRAP, total phenolics, total flavonoids.

INTRODUCTION

Interest in search of new natural antioxidants has grown over the past years as reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to chronic diseases such as cancer, cardiovascular disease, osteoporosis, and neurodegenerative diseases. Such natural antioxidant substances are believed to play a potential role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems [1]. External supply of antioxidants may overcome the effect of free radicals on the body, and in turn can prevent the occurrence of many diseases [2]. The natural sources are much safer to use due to less toxicity and side effects [3]. Hence, considerable attention has already been focused on the isolation, characterization and utilization of natural antioxidants as potential disease preventing agents.

Mahonia leschenaultia takeda, belonging to family *Berberidaceae*, is a shrub with rough, greyish-brown, corky bark. It is locally called *Thovari* by the *Todas*, the Nilgiri tribe. The paste of the stem bark is used by them in postnatal treatment in women [4]. Methanol extract of *M. leschenaultia* fruits were found to possess high level of anthocyanin, ascorbic acid, total phenolics and flavonoids and high antioxidant activity [5]. *In vivo* antioxidant and nephroprotective activities of ethanol extract of *M. leschenaultia* on acetaminophen induced toxicity in rats were studied previously [6]. However, there has been no detailed study on the *in vitro* antioxidant activity of stem and bark of *M. leschenaultia*. The objective of this study was to explore antioxidant activity, anti-lipid peroxidation activity and polyphenolic and berberine contents of methanolic extracts from bark and stem of *M. leschenaultia*.

MATERIALS AND METHODS

2.1. Instruments and Chemicals

The ultraviolet (UV) spectra were recorded on Elico SL 177, India UV-visible (UV-VIS) spectrophotometer. All chemicals including the solvents were of analytical grade; 1,1-diphenyl-2-picryl hydrazyl (DPPH), quercetin, butylated hydroxyl toluene (BHT) were purchased from Merck Chemical Co., Mumbai, India. Gallic acid was acquired from Riedel-de-Hahn, Germany.

2.2. Plant Material

The plant *M. leschenaultia* was taxonomically identified and authenticated by Dr. R. Gopalan, Taxonomist, Karpagam University, Coimbatore, India. The plant was collected from Pykara forest range, Ootacamund, Nilgiri District, Tamil Nadu, India during June 2009. The bark and stem was washed in tap water, dried and stored until further analysis.

2.3. Preparation of Extract

The bark and stem samples (100 g) were shade dried and mechanically powdered separately to obtain a course powder, which was then subjected to extraction in a Soxhlet apparatus with 5 times its volume of methanol (1:5 v/v). The extracts were concentrated *in vacuo* at 40 ± 1^0 C by rotary flash evaporator (Buchi type Rota vapor, Switzerland) under reduced pressure to obtain the dry extracts. The dry extracts were re-dissolved in methanol and the stock solutions was kept at -4^0 C to protect from light until further use. The stock solutions were used to determine total phenolics, total flavonoids, total flavonols and antioxidant capacity.

2.4. Total Phenolic Content

The total phenolic content in the methanolic extract of *M. leschenaultia* was determined using Folin-Ciocalteu phenol reagent method [7]. Briefly, diluted extract (I mL) was added with diluted Folin-Ciocalteau reagent (1 N, 1 mL). After 3 min of reaction, Sodium carbonate (Na_2CO_3) (35%, 2 mL) was added and the mixture was incubated for 30 min at room temperature. The absorbance was read at 765 nm using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The analyses were performed in triplicates. The total phenolic content was expressed as mg gallic acid equivalents from a gallic acid standard curve (mg GAE/100 g fresh mass, R^2 = 0.9968).

2.5. Total Flavonoid Content

The determination of total flavonoid content in the *M. leschenaultia* extract was based on the method reported previously [8]. The absorbance was measured at 510 nm using UV-Vis spectrophotometer with reference standard prepared with quercetin concentrations. The analyses were performed in triplicate. The total flavonoid content was estimated from a quercetin standard curve and the results were expressed as mg quercetin equivalents (mg QE/100 g fresh material, R^2 = 0.9665).

2.6. Total flavonol Content

Total flavonols in the plant extracts were estimated using the method described previously [9]. To 2.0 mL of sample (standard), 2.0 mL of 2% $AlCl_3$ in ethanol (2%) and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/ ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0255x, R^2 = 0.9812, where x was the absorbance and was the quercetin equivalent (mg/g).

2.7. Scavenging Capacity towards DPPH· Stable Radical

The determination of DPPH· scavenging activity of the *M. leschenaultia* extract was based on the method as described previously [10]. Briefly, 1 mL of aliquots of the extract and standards (20-100 µg mL^{-1}) was added to

MeOH solution of DPPH· (5 mL, 0.1 mM) and vortexed. After 20 min reaction at 25°C, the absorbance was measured at 517 nm against a blank in a UV-Vis spectrophotometer (Elico, India). BHT was used for comparison. The percentage quenching of DPPH· was calculated as follows: Inhibition of DPPH· (%) = 1-$Sample_{517nm}$/$Control_{517nm}$x100, where, $Sample_{517nm}$ was absorbance of the sample and $Control_{517nm}$ was absorbance of control. The results were expressed as EC_{50}, which means the concentration at which DPPH· radicals were quenched by 50%.

2.8. Scavenging Capacity towards Hydroxyl ion (˙OH)

Hydroxyl radicals (˙OH) were generated by a fenton reaction model system, and the scavenging capacity towards the ˙OH radical was measured using deoxyribose method with minor modifications [11]. To 1 mL of extracts (50-250 µg mL^{-1}), 1 mL of phosphate buffer (50 mM; pH 7), 0.2 mL of EDTA (1.04 mM), 0.2 mL of $FeCl_3$. $6H_2O$ (1.0 mM) and 0.2 mL of 2-deoxy-D-ribose (60 mM) were added. Following incubation in a water bath at 37°C for 60 min, 2 mL of cold TBA (in 50 mM NaOH) and 2 mL of TCA (25% w/v aqueous solution) were added to the reaction mixture. The mixture was then incubated at 100°C for 15 min. After cooling, the absorbance of the pink chromogen developed was recorded at 532 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of ˙OH was calculated as follows: Inhibition of OH˙ (%) = 1-$Sample_{532nm}$/$Control_{532nm}$x100, where, $Sample_{532nm}$ was absorbance of the sample and $Control_{532nm}$ was absorbance of control.

2.9. Scavenging Capacity towards Nitric Oxide Radical (NO)

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction with minor changes [12]. The reaction mixture (3 mL) containing SNP (10mM, 2 mL), phosphate buffer saline (0.5 mL) and the methanol extracts at different concentrations and standards (50-250 µg mL^{-1}) were incubated at 25º C for 150 min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-1-naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25º C. The absorbance of pink coloured chromophore formed during diazotization was immediately measured at 540 nm in a UV-Vis spectrophotometer. BHT was used for comparison. The percentage scavenging of NO was calculated as follows: Inhibition of NO (%) = 1-$Sample_{540nm}$/$Control_{540nm}$x100, where, $Sample_{540nm}$ was absorbance of the sample and $Control_{540nm}$ was absorbance of control.

2.10. Scavenging Capacity towards Hydrogen Peroxide (H_2O_2)

The H_2O_2 scavenging activity of extract was determined by the method as described previously [13]. The extracts (100-500 µg mL^{-1}) were dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µL of H_2O_2 (43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Percentage of H_2O_2 scavenging was calculated with the formula: 1-$Sample230_{230}$/$Control_{230}$ x 100.

2.11. Anti-Lipid peroxidation assay (TBARS)

A modified thiobarbituric acid-reactive species (TBARS) assay [14] was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid rich medium. Egg homogenate (0.5ml of 10% v/v) and 0.1ml of extract were added to a test tube and made up to 1ml with distilled water. 0.005ml of $FeSO_4$ (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5ml of 0.8% (w/v) TBA in 1.1%sodium dodecyl sulphate and 0.5ml 20% TCA were added and the resulting mixture was vortexed and then heated at 95°C for 60 min. if the sample have high amount of anthocyanin then to eliminate this non-MDA interference, another set of samples were treated in the same way, incubating without TBA. After cooling, 5.0ml of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532nm.Incubation of lipid peroxidation (%) by the extract was calculated according to [(1-E/C) x 100 where C is the absorbance value of the fully oxidized control and E is ($Abs532_{+TBA}$ – $Abs532-_{TBA}$)].

2.12. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the extracts was measured using FRAP assay described previously [15]. Briefly, the FRAP reagent contained 2,4,6-tripyridyl-s-triazine (TPTZ) solution (20 mM) in HCl (40 mM), $FeCl_3.6H_20$ (20 mM) and 0.3 M acetate buffer with pH 3.6. FRAP reagent (1.8 mL) mixed with 0.2 mL of test sample was incubated at 37º C for 10 min in a water bath. After incubation, the absorbance was measured

immediately at 593 nm. The calibration curve was plotted with absorbance vs concentration of $FeSO_4$ in the range of 0-1 mM and the total antioxidant activity was expressed as µmol Fe (II)/ g extract.

2.13. Reductive Capacity (RC)

The reducing capacity of the extracts was measured using the potassium ferricyanide reduction method [16]. Various concentrations of the extract and standards (25-500 µg mL^{-1}) were added to 2.5 mL of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [K_3Fe_3 (CN) $_6$] (1%) solution and vortexed. After incubation at 50°C for 20 min, 2.5 ml of TCA (10%, w/v) was added to all the tubes and centrifuged (Remi, India) at 3000 x *g* for 10 min. Afterwards, upper layer of the solution (5 mL) was mixed with deionized water (5 mL). To this, 1 mL of $FeCl_3$ (1%) was added to each test tube and incubated at 35°C for 10 min. The formation of Perl's Prussian colour was measured at 700 nm in a UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicated increasing reducing power. BHT was used for comparison.

2.14. Chelating Capacity (CC)

The Fe^{2+} chelating capacity (CC) was investigated according to the method described earlier [17]. Briefly, different concentrations of both the extracts and standards (50-250 µg mL^{-1}) were mixed $FeCl_2$ (2 mM) and ferrozine (5 mM). The mixture was made into 0.8 mL with deionized water. After 10 min incubation at room temperature, the absorbance of ferrous ion-ferrozine complex was measured at 562 nm in UV-Vis spectrophotometer. EDTA was used as reference standard. The percentage of chelation was calculated as follows:

Ferrous ion chelating capacity (%) = 1-$Sample_{562nm}$/$Control_{562nm}$x100,

Where, $Sample_{562nm}$ was absorbance of the sample and $Control_{562nm}$ was absorbance of control.

2.15. HPLC analysis

The berberine content was identified by using analytical HPLC method. The analytical HPLC system (P-40000, Thermo separation products, USA) employed consisted of a quaternary HPLC pump, photodiode array detector (UV 6000 LP) and a recorder. HPLC analysis was performed using a water prevail C18 analytical column (15 cm × 4.6 mm id, 5µm particle size; ALTech, IL, USA) was used. The auto-injection system (spectra system-AS 3000) consisted of a 20 µl sample loop. The mobile phase consisted of 20% acetonitrile in aqueous formic acid (v/v). The Peak responses were detected at 280 nm using UV detector. In both cases, a flow rate of 1.0 mL/min was maintained. Identification and peak assignment of the compound was based on comparison of its retention time with corresponding standard and by spiking of sample with the standard. Quantification of the compound was done using total peak area and each peak with external standard.

2.16. Statistical analysis of data

The experimental data were reported as mean ± S.D of three parallel measurements. Linear regression analysis was performed quoting the correlation coefficient.

RESULTS

The total phenol content of bark was 534.61 ± 1.040 mg gallic acid equivalents (GAE)/100 g dry matter and that of stem was 525.58 ± 0.644 mg gallic acid equivalents (GAE)/100g. Total flavonoid content of bark was 431.72 ± 0.946 mg QE/100g of dry matter and that of stem was 308.2 ± 0.917 mg quercetin equivalents (QE)/100g. The content total flavonol in bark extract was found to be 534.63 ± 0.720 mg quercetin equivalents (QE)/100g DM and that in stem was 336.33 ± 0.544 mg QE/100g (Table 1).

Table 1. Total phenol, flavonoid and flavonol contents of *M. leschenaultia* extracts

Polyphenol	Bark Extract	Stem Extract
Total Phenol[a]	534.61 ± 1.040	525.58 ± 0.644
Total Flavonoid[b]	431.72 ± 0.946	308.20 ± 0.917
Total Flavonol[b]	534.63 ± 0.720	336.33 ± 0.544

Each value in the table was obtained by calculating the average of three experiments (n=3), ± standard deviation. [a] mg gallic acid equivalents (GAE)/100g dry weight, [b] mg quercetin equivalents (QE)/100g dry weight.

The DPPH free radical scavenging ability increased sharply with the increase in concentration of the methanolic extract. Based upon the measured EC_{50} values, the DPPH· quenching ability of *M. leschenaultia* bark, stem and the standard (BHT) was found to be 40 ± 1.34 µg mL^{-1}, 50 ± 1.10 µgmL^{-1} and 26.12 ± 0.04 µg mL^{-1} respectively (Table 2).

As illustrated in Table 2, the extracts were capable of inhibiting $OH^{\bullet}$ radical formation in concentration dependent manner. The EC_{50} value of bark (190 ± 0.89 µg mL^{-1}) and stem (150 ± 1.40 µg mL^{-1}) extracts were significantly ($P<0.05$) lesser than that of BHT (16.44 ± 0.04) (Table 2).

As shown in table 2, The NO scavenging capacity was depended on concentration of the extracts. Based on the EC_{50} values, the scavenging capacity of bark extract (110 ± 1.21 µg mL^{-1}) was significantly lower than that of stem extract (90 ± 1.15 µg mL^{-1}). The EC_{50} value of BHT was found to be 46.34 ± 0.08 µgmL^{-1} (Table 2).

Table 2 displays a concentration dependent inhibition of H_2O_2 by bark and stem extracts. Based on the EC_{50} values, the scavenging capacity of bark extract (100 ± 1.25 µgmL^{-1}) was significantly ($P<0.05$) higher than that of stem extract (150 ± 1.03 µg mL^{-1}). The EC_{50} value of BHT was found to be 65 ± 0.254 (Table 2).

The inhibitory effect of bark and stem extracts and BHT on TBARS production in egg yolk homogenate induced by $FeSO_4$ is shown in Table 2. Both extracts were capable of preventing the formation of MDA in a dose dependent manner. But the bark (EC_{50} 80 ± 1.16 µg mL^{-1}) and stem (EC_{50} 100 ± 0.92 µg mL^{-1}) extracts showed less prevention towards generation of lipid peroxide than BHT (EC_{50} 43.87 ± 2.02 µg mL^{-1}). Figure 1 shows the percentage anti-lipid peroxidation (ALP %) potential of the extracts with their corresponding BHT equivalence.

Table 2. Antioxidant activity of bark and stem extracts of *M. leschenaultia*

	EC_{50} (µg mL^{-1})[a]		
Assays	Bark Extract[b]	Stem Extract[b]	BHT[b]
DPPH	40.32 ± 1.34	50.34 ± 1.10	26.12 ± 0.04
$OH^{\cdot}$	190.11 ± 0.89	150.56 ± 1.40	16.44 ± 0.04
NO	110.44 ± 1.21	90.98 ± 1.15	46.34 ± 0.08
H_2O_2	100.23 ± 1.25	150.12 ± 1.03	65.00 ± 0.25
ALPO	80.00 ± 1.16	100.00 ± 0.92	43.87 ± 2.02

[a]EC_{50} value: the effective concentration at which the antioxidant capacity was 50%. EC_{50} was obtained by interpolation from linear regression analysis. [b]Data are mean ± standard deviation (n = 3).

The FRAP values of bark and stem extracts of *M. leschenaultia* are summarized in Table 3. The FRAP value of bark extract (175 µm Fe^{2+} /100g sample) was comparatively higher than that of stem extract (120 µm Fe^{2+} /100g sample) at the concentration of 250 µg/ ml ($P<0.05$).

Table 3. FRAP value of bark and stem extracts of *M. leschenaultia*

	FRAP (µm Fe^{2+}/ 100g Dry Matter)[a]	
Concentration (µg/ml)	Bark Extract	Stem Extract
50	20.24 ± 0.44	5.32 ± 0.08
100	61.54 ± 0.04	25.67 ± 0.04
150	100.20 ± 0.54	55.42 ± 0.24
200	140.21 ± 0.02	95.87 ± 0.42
250	175.80 ± 1.02	120.21 ± 0.22

[a]Data expressed as mean ± SD of three individual experiments (n = 3)

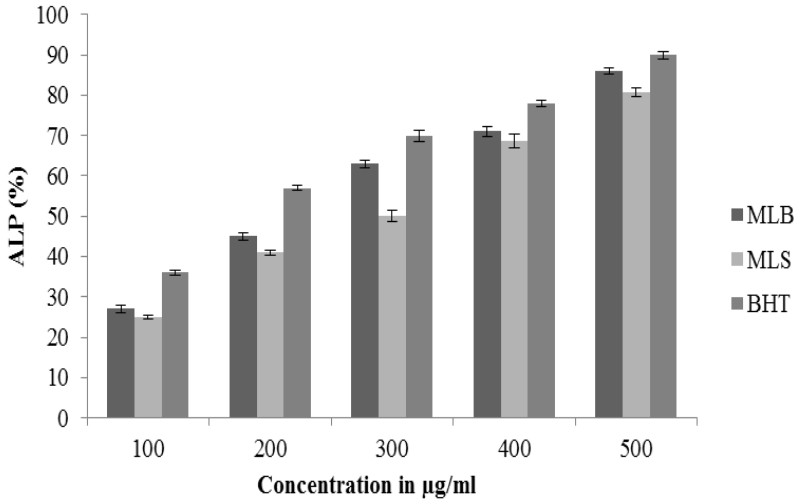

Figure 1. ALP % of methanol extracts of bark and stem of *M. leschenaultia*

The reduction of ferrous ion (Fe3+) to ferric ion (Fe2+) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm. Figure 2 shows the concentration dependent increase in reducing capacity in terms of absorbance values at 700 nm. The reducing power of the bark extract (0.471 ± 0.013) and stem extract (0.428 ± 0.014) at a dosage of 100 µg/mL was found to be below that of BHT (0.688 ± 0.044).

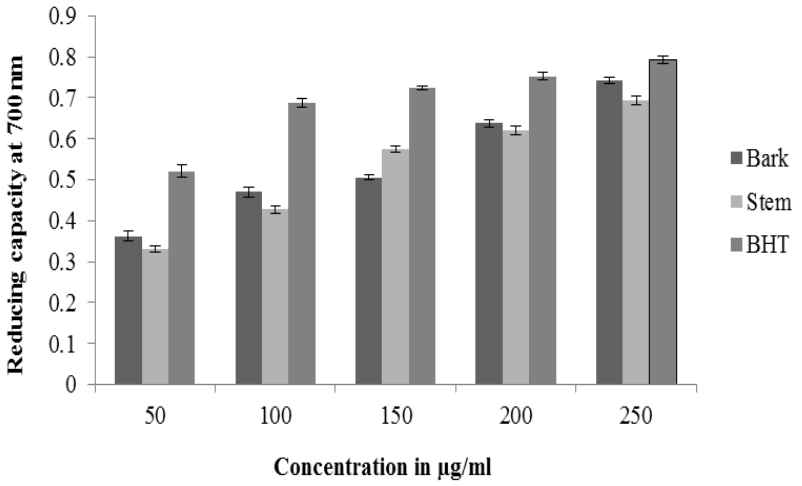

Figure 2. Reducing capacity of methanol extracts of bark and stem of *M. leschenaultia*

The methanol extract of *M. leschenaultia* revealed a concentration dependent increase in chelating capacity (Figure 3). The iron chelating capacity of bark extract, stem extract and positive control were found to be 31%, 25.5 % and 55.63% at a concentration of 100µg/ml.

HPLC method enabled qualitative and quantitative analysis of Berberine in *M. leschenaultia*. From the calibration curve results, the amount of Berberine, in the sample injected was calculated. The amount of Berberine in bark (0.97 mg/kg) was similar to that in stem (0.98 mg/kg). The chromatogram of Berberine standard, stem and bark are given as Figure 4, Figure 5 and Figure 6 respectively. Peak 1 in both figure 1 and figure 2 represents Berberine.

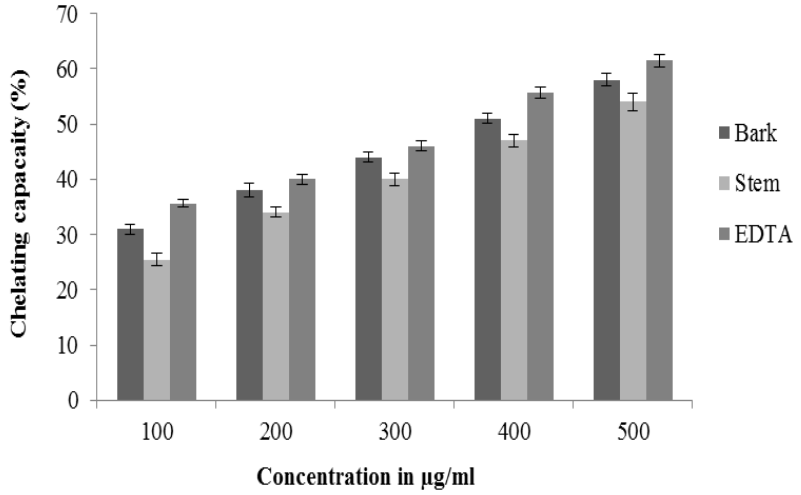

Figure 3. Chelating capacity of methanol extracts of bark and stem of *M. leschenaultia*

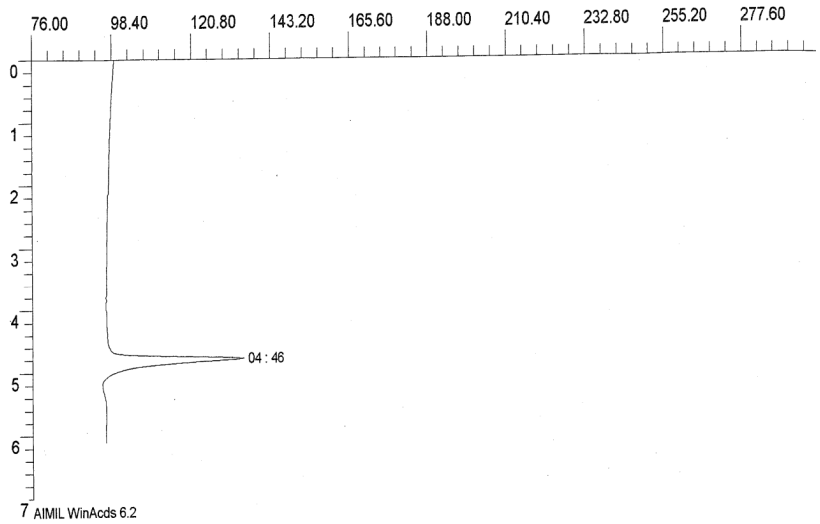

Figure 4. HPLC analysis of Berberine standard

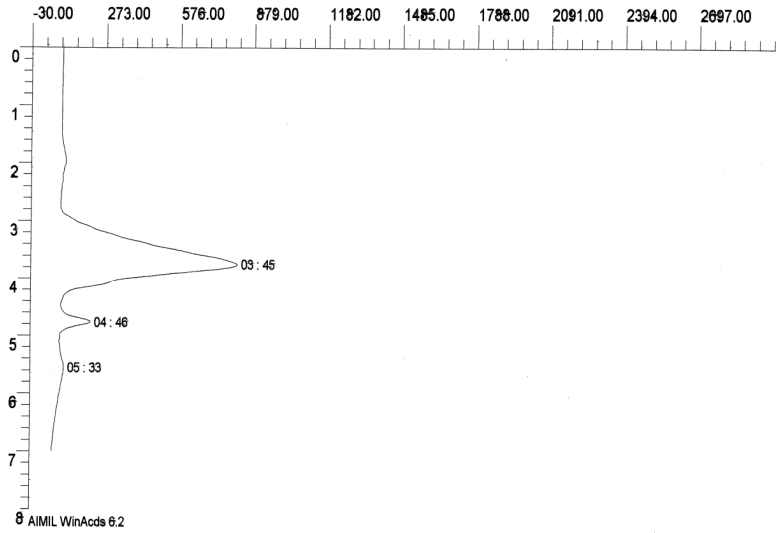

Figure 5. HPLC analysis of *M. leschenaultia* stem extract

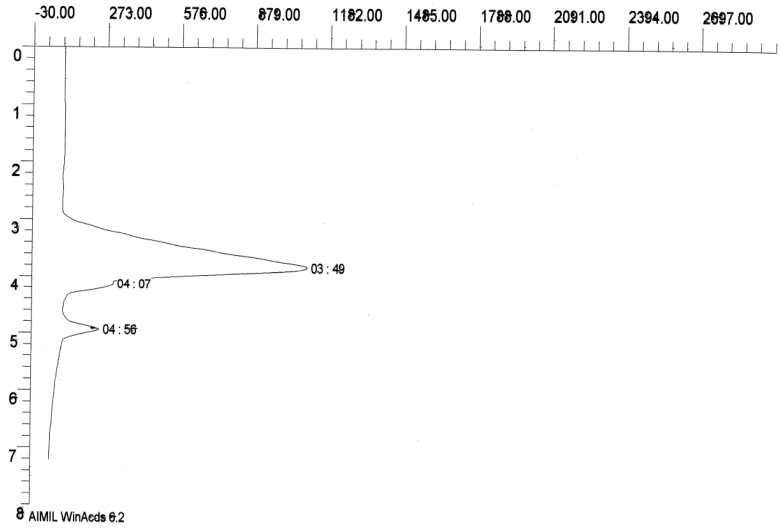

Figure 6. HPLC analysis of *M. leschenaultia* bark extract

Parameters	Stem Extract	Bark Extract	Standard
RT (min/sec)	3:45	3:50	4:47
Content (mg/kg)	0.98	0.97	0.98

The TPC of bark extract exhibited an apparent linear relationship with DPPH radical scavenging activity (r^2 = 0.98), nitric oxide radical scavenging activity (r^2 = 0.97), hydrogen peroxide scavenging activity (r^2 = 0.98), reducing capacity (r^2 = 0.97) and hydroxyl scavenging activity (r^2 = 0.96). The TPC of stem extract also exhibited linear relationship with DPPH radical scavenging activity (r^2 = 0.99), nitric oxide radical scavenging activity (r^2 = 0.99), hydrogen peroxide scavenging activity (r^2 = 0.98), reducing capacity (r^2 = 0.96) and hydroxyl scavenging activity (r^2 = 0.97). The TFC of bark was well correlated with DPPH radical scavenging activity (r^2 = 0.98), reducing power (r^2 = 0.97), hydroxyl radical scavenging activity (r^2 = 0.96), nitric oxide scavenging activity (r^2 = 0.97) and hydrogen peroxide scavenging activity (r^2 = 0.98).

DISCUSSION

The antioxidant activity of medicinal plants is mainly related to their phenolic compounds [18]. Polyphenol are the major plant compounds and are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Their antioxidant activity is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers [19].

In the view of the up surging interest in the health benefits of the medicinal plants, we examined total phenolics, flavonoids and flavonols and evaluated the antioxidant properties of bark and stem extracts of *M. leschenaultia*. The bark extract features a substantial content of total phenolics as compared with stem extract. The total phenolic content was comparatively higher than the total flavonoid and flavonol contents. The total phenolic content in bark and stem of *M. leschenaultia* is higher when compared to 70% aqueous methanolic extracts of its fruits [5]. Previous reports revealed that methanol extract of *M. leschenaultia* bark and stem extracts possess more content of flavonoids than its fruit extract [5].

DPPH is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [20]. It was found that the radical scavenging activities of bark and stem extracts increased with increasing concentration. The bark extract possessed more hydrogen donating ability than the stem extract and it was comparable to that of BHT. The DPPH scavenging activity of bark and stem was higher than that exhibited by its fruit extract [5]. The DPPH scavenging capacity of the plant extracts may be related to the phenolic compounds present [21].

Hydroxyl radical ($^{\cdot}OH$), the most reactive free radical, has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity [22]. In this study, *M. leschenaultia* extracts inhibited the degradation of 2-deoxyribose in a dose dependent manner. The bark extract exhibited higher activity than the stem extract, but the hydroxyl radical scavenging activity of both the extracts was lower than that of BHT. Earlier studies showed that bark and stem extracts of *M. leschenaultia* hold low activity than the fruit extracts of some Berberidaceae members [23].

In addition to reactive oxygen species, NO is also implicated in chronic inflammation, cancer and other pathological conditions. The NO generated from SNP at physiological pH reacts with oxygen (O_2) to form nitrite ions. The methanol extracts of *M. leschenaultia* competed with O_2 to react with nitrite ions and thus inhibits the NO generation. Here, the stem extract possessed higher scavenging activities than the bark extract. Here also the bark and the stem extracts showed lesser scavenging activity than that of BHT. Fruit extracts of some members of the family Berberidaceae showed more NO scavenging activity than bark and stem of *M. leschenaultia* [23].

Hydrogen peroxide is an important ROS formed *in vivo* by many oxidizing enzymes such as superoxide dismutase. It has a strong oxidizing property with the ability to penetrate biological membranes. Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cells when it gives rise to hydroxyl radical in the cells [24]. The bark and stem extracts of *M. leschenaultia* were capable of scavenging H_2O_2 in a concentration dependent manner. Bark of *M. leschenaultia* showed more scavenging capacity than the stem.

Lipid peroxidation, which is widely recognized as primary toxicological event, is caused by the generation of free radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds. The TBARS assay has been used to measure the degree of lipid peroxidation. TBA reacts specifically with malondialdehyde (MDA), a secondary product of lipid peroxidation to give a red chromogen, which may then be determined spectrophotometrically [25]. In this study, both bark and stem extracts were capable of preventing the formation of MDA in a dose dependent manner. But the highest anti-lipid peroxidation activity was revealed by the bark extract of *M. leschenaultia* than the stem extract. This assay revealed that the extracts might prevent reactive radical species from damaging biomolecules such as lipoprotein, DNA, amino acids, sugar, proteins and PUFA in biological and food systems.

The ability of plant extracts to reduce Fe^{3+}/Fe^{2+} was determined by FRAP assay. FRAP assay measures the reducing capacity by increased sample absorbance based on the formed ferrous ions, and the assay may not be complete even several hours after the reaction starts, such that a single end-point of the reaction cannot be determined [26,27].The change in absorbance at 593 nm owing to the formation of blue coloured Fe^{2+} - TPTZ complex from the colourless oxidized Fe^{3+} form by the action of electron donating antioxidants [28]. The FRAP value of bark extract was found to be higher than that of the stem extract. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidants present, thus it can be reported that extracts of *M. leschenaultia* may act as free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

In the reducing power assay, the presence of antioxidants in the bark and stem extracts of *M. leschenaultia* was able to convert the oxidized form of Fe^{3+} into Fe^{2+} which was measured by the intensity of the resultant Prussian blue color complex. With the increase of concentration, the absorbance of the extracts and the standard were found to be increased gradually [29]. Bark extract showed high reducing capacity than the stem extract and the reducing capacities of both bark and stem was lesser than that of BHT. Earlier reports suggests that bark and stem of *M. leschenaultia* holds low reducing capacity than fruits of some members of the same family [23]. The reducing capacity is generally associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom [30]. The result indicated that the marked reducing power of the bark and stem extracts seems to be due to the presence of polyphenols which may act in a similar fashion as reductones.

The chelating activities for Fe^{2+} of the extracts were assessed by the inhibition of formation of red-colored ferrozine and ferrous complex. The formation of the red complex was inhibited concentration-dependently in the presence of extracts. The data obtained from this assay demonstrated that the extracts of *M. leschenaultia* revealed an effective capacity for metal-binding, suggesting that they may play a protective role against oxidative damage by sequestering Fe^{2+} ions. But the chelating capacity of *M. leschenaultia* was found to be lesser than *Berberis tinctoria* [22].

It is widely accepted that the antioxidant activity of a plant extract is correlated to its phenolic content [31]. In the present study, strong correlations were observed between phenolic content and antioxidant activity. According to recent reports, the strong scavenging capacity of the extracts on DPPH, hydroxyl, nitric oxide, hydrogen peroxide radicals might possibly due to the phenolic compounds which could act as a hydrogen donor antioxidant. The antioxidant potential of polyphenols has been correlated to the capacity of donating hydrogen. The number and the configuration of H-donating hydroxyl groups are both important structural features influencing the antioxidant capacity of phenolic compounds. [32]. Therefore, it was considered that the high antioxidant capacity of the bark and stem extract of *M. leschenaultia* could be attributable to its high amount of polyphenolic content.

Berberine is a quaternary ammonium salt from the proto-berberine group of isoquinoline alkaloids. It is found in such plants as *Berberis aquifolium*, *Berberis vulgaris* and *Tinospora cordifolia* and to a smaller extent in *Argemone mexicana* and *Eschscholzia californica*. Berberine is usually found in the roots, rhizomes, stems and barks [33]. During the last few decades, many studies have shown berberine has various beneficial effects on the cardiovascular system and significant anti-inflammatory [34] and antimicrobial [35] activities. It exerts up-regulating activity on both low-density-lipoprotein receptor (LDLR) and insulin receptor (InsR). This one-drug-multiple-target characteristic might be suitable for the treatment of metabolic syndrome [36]. Berberine has been tested and used successfully in experimental and human diabetes mellitus [37]. In the present study, HPLC analysis of bark and stem extracts of *M. leschenaultia* revealed the presence of berberine. So the plant could act as a potent source of this multiple target alkaloid which can be used for impending drug development [38].

CONCLUSION

In this study we focused on antioxidant properties of bark and stem of *M. leschenaultia*. This study demonstrated that *M. leschenaultia* bark and stem extracts efficiently scavenged DPPH, $OH^{\cdot}$, NO and H_2O_2 *in vitro*. The plant showed good chelating capacity, reducing capacity and anti-lipid peroxidation activity. These activities of the plant were strongly correlated with its phenolic and flavonoid contents. HPLC analysis of the extracts revealed the presence of an important alkaloid Berberine, which is very frequently used in traditional medicinal system. As a result, bark and stem extracts of *M. leschenaultia* seem to be good sources of natural antioxidants.

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