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In vitro antioxidant activity of *Amaranthus lividus* L.

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ABSTRACT

Water, methanol and ethyl acetate extracts from stems with leaves and flowers of *Amaranthus lividus* L., one of the most popular leafy vegetable consumed in the west Black Sea region of Turkey, were tested *in vitro* for their ability to inhibit peroxidation of phosphatidylcholine liposomes induced with Fe^{3+}/ascorbate, to scavenge $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and hydroxyl radicals, to reduce Fe (III) to Fe (II) and to chelate Fe (II) ions. The results showed that amaranth vegetable contained naturally occuring antioxidant components and possessed antioxidant activity which may be attributed to its lipid peroxidation inhibitory, radical scavenging and metal chelating activities. The antioxidant activities of the water and ethyl acetate extracts were not concomitant with the development of their reducing power. It was concluded that *A. lividus* might be a potential source of antioxidants.

1. Introduction

Interest in the search for new natural antioxidants has grown over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to diseases such as cancer, cardiovascular disease, osteoporosis, and degenerative 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 (Halliwell & Gutteridge, 1984).

Amaranthus plants (Amaranthaceae) are spread throughout the world, growing under a wide range of climatic conditions and they are known to infest or to produce useful feed and food products (Rastrelli, Pizza, Saturnino, Schettino, & Dini, 1995). The leaves of amaranth constitute an inexpensive and rich source of protein, carotenoids, vitamin C, and dietary fibre (Shukla et al., 2006), minerals like calcium, iron, zinc, magnesium (Kadoshnikov, Kadoshnikova, Kulikov, & Martirosyan, 2008; Shukla et al., 2006), and phosphorus (Ozbucak, Ergen Akcin, & Yalcin, 2007).

Limited studies have been reported on the antioxidant activity of *Amaranthus* leaf extracts. The antioxidant properties and phenolic content of the fresh and blanched green leafy vegetables, including other *Amaranthus* species, have been studied (Amin, Norazaidah, & Emmy Hainida, 2006; Oboh, 2005; Pacifico et al., 2008; Saxena, Venkaiah, Anitha, Venu, & Raghunath, 2007).

A. lividus L. *(=A. blitum),* locally known as "dari mancari", is one of the most popular leafy vegetables consumed in west Black Sea region of Turkey. The objectives of this study were to evaluate the antioxidant activity and antioxidant components of *A. lividus* extracts obtained from three solvents. The extraction method of the antioxidants affects the total phenolic contents and antioxidant capacities of the extracts. In this study, the extraction efficiency of the traditional boiling water extraction method was studied by comparing its antioxidant activity and phenolic content to extraction methods using methanol and ethyl acetate. The antioxidant activities of *A. lividus* extracts were evaluated based on the ability of the extracts to inhibit lipid peroxidation in phosphatidylcholine liposomes induced by Fe^{3+}/ascorbate system, to scavenge $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and hydroxyl radicals, to reduce Fe (III) to Fe (II) and to bind to Fe (II) ions. The results were compared to those of gallic acid and BHA.

2. Materials and methods

2.1. Chemicals

2-Deoxy-D-ribose, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{\cdot+}$) and 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2,2-Diphenyl-1-picryl-hydrazyl ($DPPH^{\cdot}$), bathophenanthroline, lecithin [soybean L-α-phosphatidylcholine (Type IV-S)], α-tocopherol and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), thiobarbituric acid (TBA), m-phosphoric acid, o-phosphoric acid, pyrogallol, L-ascorbic acid, ferrous

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and ferric chloride and 2,6-dichlorophenol-indophenol natrium were obtained from Merck. All other reagents were of analytical grade.

2.2. Plant material

The stems with leaves and flowers of *A. lividus* L. were collected in August from Bartin, Turkey, and identified by Prof. Dr. Asuman Baytop. A voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, Istanbul University (ISTE); herbarium code number: ISTE 83401. Plant materials were washed with distilled water and dried at room temperature. The dried stems with leaves and flowers were manually ground to a fine powder.

2.3. Preparation of extracts

A crude distilled water extract was prepared by heating powdered *A. lividus* (10 g) in a flask with 100 ml distilled water for 30 min whilst stirring. Similarly, 10 g of powdered *A. lividus* was extracted successively with methanol or ethyl acetate in a Soxhlet apparatus for 4 h. The extracts were filtered and evaporated to dryness under reduced pressure and controlled temperature (40–50 °C) in a rotary evaporator, then weighed to determine the total extractable compounds (EC). The water extract yielded a dark-brown solid residue weighing 1.30 g (13.0% w/w), and the methanol and the ethyl acetate extracts yielded semi-solid residues weighing 1.16 g (11.6% w/w) and 0.2 g (2% w/w), respectively. All the extracts were kept at −20 °C and were dissolved in water or solvent before use.

2.4. Determination of the antioxidant components

Total soluble phenolics in the water, methanol and ethyl acetate extracts of stems, leaves, and flowers of *A. lividus* were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977) with some modifications. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid standard solution (covering the concentration range between 0.05 and 0.4 mg/ml) and expressed as mg gallic acid/g dry weight (DW) of the plant material. Data were presented as the average of triplicate analyses.

β-Carotene was determined according to the method of Nagata and Yamashita (1992). The contents of α-tocopherol were estimated by the method of Desai (1984). Ascorbic acid was determined according to the method of Omaye, Turnbull, and Sauberlich (1979).

2.5. Antioxidative effect on liposome peroxidation

The antioxidant activity of the extracts was evaluated by the ability of different concentrations of plant extracts to inhibit lipid peroxidation in liposomes, induced by $FeCl_3$/ascorbate system. This lipid peroxidation assay was based on the method described by Duh, Tu, and Yen (1999) with some modifications.

Lecithin (300 mg) was suspended in 30 ml of 10 mmol/l phosphate buffer (pH 7.4). This suspension was then sonicated with a rod using an ultrasonic homogenizer (Bandelin, Berlin, Germany) at 30 s intervals for 10 min until an opalescent suspension was obtained.

The sonicated solution (10 mg/ml), $FeCl_3$, ascorbic acid and plant extracts (2.5–60 mg/ml water, 2.5–40 mg/ml methanol or 1.25–20 mg/ml ethyl acetate extracts) or reference antioxidants (0.31–5 mg/ml gallic acid or 0.625–40 μg/ml BHA) were mixed to produce a final concentration of 3.08 mg liposome/ml, 123 μmol/l $FeCl_3$ and 123 μmol/l ascorbic acid. After 1 h incubation at 37°C, the formation of lipid peroxidation products was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) on the basis that malondialdehyde (MDA) reacted with thiobarbituric acid at 532 nm according to Buege and Aust (1978).

2.6. Total radical antioxidant potential method

The total radical antioxidant potential of the extracts was measured using the Trolox equivalent antioxidant coefficient (TEAC) assay as described by Re et al. (1999). The total antioxidant capacity value in a sample was assessed as TEAC. The TEAC values were calculated by using a regression equation between the Trolox concentration and the percentage of inhibition of absorbance at 734 nm at 6 min of incubation and were expressed as mmol TEAC per gram of DW.

2.7. DPPH radical scavenging activity

The DPPH· scavenging activity of the extracts from *A. lividus* was measured according to the procedure described by Brand-Williams, Cuvelier, and Berset (1995).

2.8. Hydroxyl radical scavenging activity

The effect of extracts on hydroxyl radicals was assayed by using the deoxyribose method (Nagai, Myoda, & Nagashima, 2005).

2.9. Reducing power

In the reducing power assay, the presence of reductants (antioxidants) in the samples results in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. The reducing powers of the extracts from *A. lividus* stems with leaves and flowers, gallic acid and BHA were determined according to the method described by Chung, Chen, Hsu, Chang, and Chou (2005).

2.10. Iron (II) chelation activity

The chelating of ferrous ions by the extracts from stems with leaves and flowers of *A. lividus* and reference antioxidant was estimated by the method of Dinis, Madeira, and Almeida (1994).

2.11. Statistical analysis

Results were expressed as the mean ± the standard deviation of triplicate analysis. Statistical comparisons were performed using the Student's *t*-test. Differences were considered significant at $p < 0.05$. The correlation coefficient (r^2) between the parameters tested was established by regression analysis.

3. Results and discussion

3.1. Extract yield (amount of total extractable compounds) and contents of total phenolics, β-carotene, α-tocopherol, and ascorbic acid

Water, methanol and ethyl acetate extracts prepared from 10 g *A. lividus* gave a yield of 0.130, 0.116, and 0.020 g extractable compounds (EC) per gram of DW, respectively (Table 1).

Extracts contained only a very low amount of phenolic compounds. The content of total phenolic compounds is given in Table 1. The method using ethyl acetate showed a greater efficiency in the extraction of phenolic compounds than that with water and methanol. The mean of the total phenolic content per gram of crude extract of ethyl acetate extract (22.8 ± 1.91 mg GAE/g) was found to be higher than that of the water (12.0 ± 0.76 mg GAE/g)

Table 1
Total extractable compounds (EC), total phenolic compounds (PC) (as gallic acid equivalents) and contents of β-carotene, α-tocopherol and ascorbic acid in water, methanol and ethyl acetate extracts from *A. lividus*. Values were the means of three replicates ± standard deviation.

Extract	EC (g/g DW)	PC (mg/g DW)	PC/EC (%)	β-Carotene (mg/g DW)	α-Tocopherol (μg/g DW)	Ascorbic acid (mg/g DW)
Water	0.130	1.55[a] ± 0.098	1.19	ND	ND	0.191[a] ± 0.007
Methanol	0.116	1.51[a] ± 0.130	1.3	1.24[a] ± 0.020	31.43[a] ± 0.92	0.196[a] ± 0.014
Ethyl acetate	0.020	0.46[b] ± 0.039	2.3	0.37[b] ± 0.013	7.12[b] ± 0.15	ND

ND: Not detected.
[a,b] Values with different letters in the same column were significantly ($p < 0.05$) different.

and methanol (13.0 ± 1.18 mg GAE/g) extracts. However, the extraction with ethyl acetate resulted in lower yields of extractable compounds. The amounts of total phenolic compounds per gram of DW in extracts obtained with water (1.55 ± 0.098 mg GAE/g DW) and methanol (1.51 ± 0.13 mg GAE/g DW) were higher than the amounts obtained with ethyl acetate (0.46 ± 0.039 mg GAE/g DW). Although the extraction yield of total extractable compounds with ethyl acetate was very small, the fraction that represented total phenolic compounds (2.3%) was twice that found for extracts obtained with water (1.19%) and methanol (1.3%). Thus, it was concluded that ethyl acetate was more efficient at extracting phenolic compounds from the plant than was water and methanol. The greater efficiency of ethyl acetate in extracting the phenolic compounds would be expected to result in higher antioxidant activity. These values were higher than that reported for leaves of *A. cruenthus* (0.3 g/100 g) (Oboh, 2005). However, values obtained in this work showed less phenolic content compared to four *Amaranthus* varieties reported by Amin et al. (2006). The fact that *A. lividus* stems with leaves and flowers were relatively low in phenolic compounds is in agreement with Saxena et al. (2007) and Pacifico et al. (2008).

Amaranthus plants have been reported as one of many vegetables rich in antioxidant components. Carotenoids, ascorbic acid, flavonoids, and phenolic acids might be some of the components able to contribute to their antioxidant activity (Amin et al., 2006). Whereas total phenols were the major antioxidant components found in the water, methanol and ethyl acetate extracts of *A. lividus*, ascorbic acid was found in small amounts in water (0.191 ± 0.007 mg/g DW) and methanol extracts (0.196 ± 0.014 mg/g DW) (Table 1) and these values were lower than that of other *Amaranthus* species (Guil, Rodriguez-Garcia, & Torija, 1997; Gupta & Prakash, 2008; Oboh, 2005). β-Carotene was detected in the ethyl acetate and methanol extracts, in the range of 0.37–1.24 mg/g DW. These values were comparable or higher than those reported by Raju, Varakumar, Lakshminarayana, Krishnakantha, and Baskaran (2007) for *A. gangeticus*, *A. tristis*, and *A. viridis* leaves and Guil et al. (1997) reported for *A. viridis* leaves.

α-Tocopherol was found only in methanol (31.4 ± 0.92 μg/g DW) and ethyl acetate (7.12 ± 0.15 μg/g DW) extracts of *A. lividus* (Table 1). Tocopherols are important biological antioxidants which prevent oxidation of body lipids, including polyunsaturated fatty acids and lipid components of cells and organelle membranes.

3.2. Antioxidative effect on liposome peroxidation

The total antioxidant activity, which reflected the ability of the extracts to inhibit the $FeCl_3$/ascorbic acid induced phosphatidylcholine liposome oxidation, was measured and compared with that of a control which contained no antioxidant component. The inhibitory effects of extracts at different concentrations on lipid peroxidation in liposomes are shown in Fig. 1. All the extracts demonstrated the ability to inhibit the formation of TBARS in a concentration dependent manner. The water extract exhibited a poor antioxidant activity of 32.3 ± 0.28% at a concentration of 20 mg/ml and high inhibition, about 94.4 ± 2.32%, only when

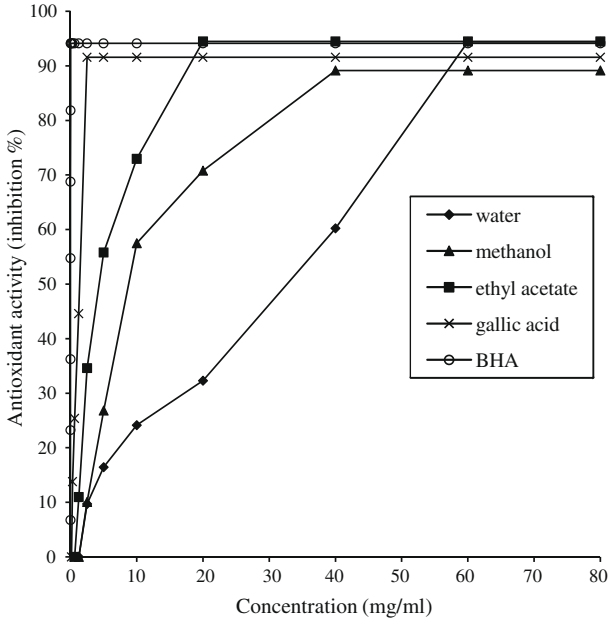

Fig. 1. The inhibitory effect of the extracts from *A. lividus* on lipid peroxidation in liposomes. Gallic acid and BHA were used as reference antioxidants. Values are means ± SD ($n = 3$).

60 mg/ml was used. The methanol extract showed an inhibition from 10.0 ± 1.20% at a concentration of 2.5 mg/ml to 89.1 ± 1.84% at a concentration of 40 mg/ml. The ethyl acetate extract showed an increase in antioxidant activity from 10.9 ± 1.49% at 1.25 mg/ml to 94.5 ± 1.49% at 20 mg/ml. The inhibitory effects of gallic acid on TBARS formation were 13.8 ± 1.15% at 0.31 mg/ml and 91.5 ± 0.70 at 2.5 mg/ml. BHA showed good inhibition of 6.7 ± 0.32 to 94.1 ± 2.09% at the concentration range between 0.625–40 μg/ml. There was no significant difference ($p > 0.05$) in antioxidant activities between the water extract at 60 mg/ml, the ethyl acetate at 20 mg/ml, the BHA at 40 μg/ml and gallic acid at 2.5 mg/ml. The results were in agreement with Lindsey, Motsei, and Jäger (2002) who reported a high antioxidant activity (90%) in an aqueous extract of boiled *Amaranthus* leaves. Kelawala and Ananthanarayan (2004) also reported that amaranth leaves (*A. gangeticus*) inhibited the auto-oxidation of linoleic acid by about 50%.

The antioxidant activity was expressed as an effective concentration at 50% (EC_{50}, effective concentration at which the antioxidant activity was 50%). The lowest EC_{50} value of 7.33 ± 0.53 mg/ml was detected in the ethyl acetate extract, followed by methanol extract (17.5 ± 0.51 mg/ml), whilst the water extract had the greatest EC_{50} value of 33.3 ± 0.98 mg/ml, suggesting that ethyl acetate is a better solvent for the extraction of antioxidant compounds from vegetable amaranth. However, when compared to BHA (11.6 ± 0.26 μg/ml) and gallic acid (1.7 ± 0.043 mg/ml), all the tested extracts showed significantly ($p < 0.05$) lower antioxidant activity. Similarly, Conforti et al. (2005) reported that methanol

extracts of two varieties of *A. caudatus* seeds did not demonstrate effective antioxidant activity whilst the ethyl acetate extract and squalene showed higher activities. Comparison of EC_{50} values of *A. lividus* with the EC_{50} values of the ethyl acetate extract of two varieties of *A. caudatus* seeds showed that *A. lividus* was the less active antioxidant.

3.3. Total radical antioxidant potential method

The $ABTS^{\cdot+}$ radical scavenging activity (%) of the water, methanol and ethyl acetate extracts of *A. lividus*, compared to gallic acid and BHA are shown in Fig. 2. They increased with increasing concentration, reaching 84.6 ± 1.16%, 81.9 ± 2.51%, and 95.5 ± 1.06%, respectively at a concentration of 60, 40 and 20 mg/ml, respectively, and these values were comparable to those of the positive controls, gallic acid (99.2 ± 0.15%), and BHA (98.4 ± 0.77%) at a concentration of 0.31 and 0.625 mg/ml, respectively.

From the effective concentration (EC_{50}) of the extracts, it was seen that the ethyl acetate extract had the highest $ABTS^{\cdot+}$ radical scavenging activity as shown by the lowest value of EC_{50}, followed by methanol extract, whilst the water extract had the least activity. However when compared to reference antioxidants, gallic acid and BHA, all the tested extracts showed significantly ($p < 0.05$) lower $ABTS^{\cdot+}$ radical scavenging activity.

The TEAC value is a quantification of the effective antioxidant activity of the extracts. The higher TEAC value implies a greater antioxidant activity. Total antioxidant potential of the extracts defined as the concentration of Trolox with the equivalent antioxidant activity as a 1 mM concentration of the tested substances, was highest for the ethyl acetate extract (2.25 ± 0.026 mM at 20 mg/ml) whilst corresponding values for water and methanol extracts were 1.98 ± 0.01 at 60 mg/ml and 1.92 ± 0.056 mM at 40 mg/ml, respectively. Rice-Evans, Miller, and Paganga (1997) reported that flavonoids with efficient scavenging properties have a TEAC value of $\geqslant$1.9 mM, in comparison to less efficient antioxidants with a TEAC value of $\leqslant$1.5 mM. Though the total phenolic content of extracts from *A. lividus* were found to be relatively low, the TEAC values of $\geqslant$1.9 mM seem to be sufficient for the extract to function as an efficient antioxidant. As a result of the low extraction yield with ethyl acetate, the extract showed the lowest TEAC per gram of DW (2.30 mM/g DW), followed by water (4.28 ± 0.025 mM/g DW) and methanol (5.62 ± 0.17 mM/g DW) extracts, in increasing order. This emphasises the importance of the extraction yield.

3.4. DPPH radical scavenging activity

The extracts showed maximum hydrogen-donating ability in the presence of DPPH stable radicals at high concentrations. As shown in Fig. 3, the scavenging activities of the extracts on DPPH radicals were similar to the results of the scavenging activities on $ABTS^{\cdot+}$ and antioxidant activities in phosphatidylcholine liposome system. Scavenging activity of the ethyl acetate (93.2 ± 0.55%) at 10 mg/ml and the methanol extract (89.9 ± 1.72%) at 40 mg/ml did not show any significant difference ($p > 0.05$) from that of gallic acid (93.3 ± 0.19%) at 0.08 mg/ml and BHA (93.6 ± 0.22%) at 0.16 mg/ml. The scavenging activity of the water extract was significantly ($p < 0.05$) lower (12.1 ± 0.93%) at 10 mg/ml and, at a concentration of 60 mg/ml, reached a plateau of 75.5 ± 0.16%. These results were consistent with the previous observation that *Amaranthus* varieties (Oboh, 2005) contained radical scavenging agents that could directly react with and quench stable DPPH radicals. The ability of an *A. paniculatus* extract to act as a free radical scavenger or hydrogen donor was reported (Amin et al., 2006). Moreover, the antioxidative properties of the ethyl acetate extract of *A. lividus* on scavenging $DPPH^{\cdot}$ were found to be superior to those of *A. cruentus* (52.4% at 25 mg/ml) (Oboh, 2005).

The DPPH scavenging activities of the extracts, expressed as an EC_{50} value, ranged from 6.75 to 42.4 mg/ml. The ethyl acetate extract exhibited the strongest antioxidant activity (6.75 ±0.083 mg/ml), followed by the methanol extract (24.8 ± 0.36 mg/ml) and the water extract (42.3 ± 0.86 mg/ml) which showed the weakest activity. The *A. lividus* extracts showed weaker DPPH radical scavenging activities compared to the DPPH radical scavenging activity of the methanolic extract of *A. retroflexus* (IC_{50} value of 92.7 μg/ml) (Pacifico et al., 2008). Cai, Sun, and Corke (2005) reported that all the tested betalains from plants in the family Amaranthaceae exhibited a strong antiradical activity (EC_{50} values ranged from 3.4 to 8.4 μM), representing a new class of dietary antioxidants.

These results suggested that the ethyl acetate extract contained the strongest free radical scavenging compounds. However, none of the extracts was as effective as a DPPH free radical scavenger

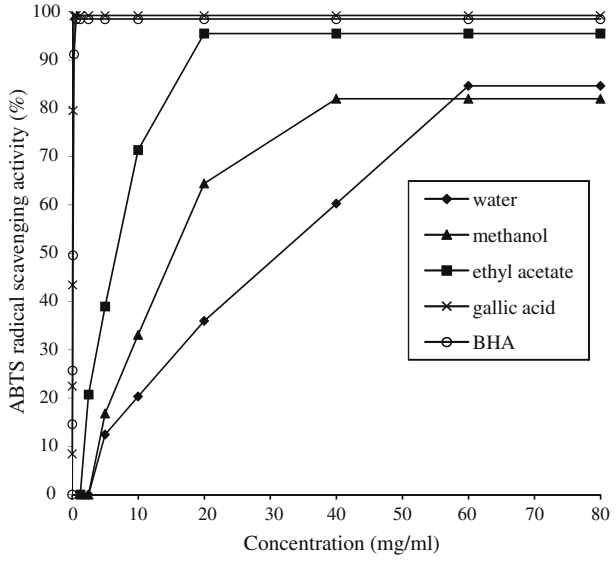

Fig. 2. ABTS radical scavenging activity of the extracts from *A. lividus*. Gallic acid and BHA were used as reference antioxidants. Values are means ± SD ($n = 3$).

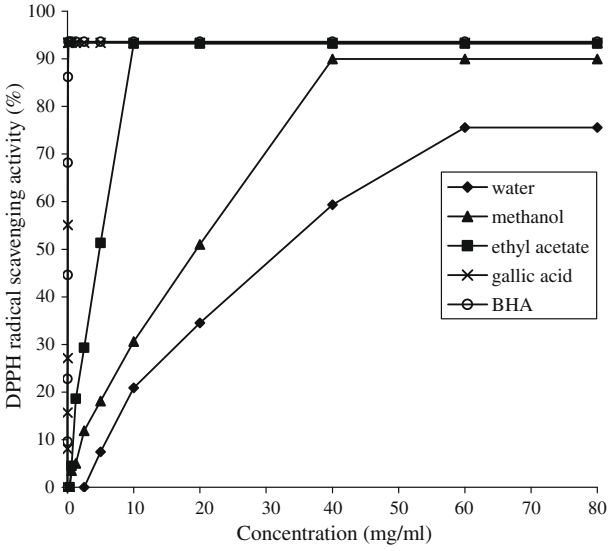

Fig. 3. DPPH radical scavenging activity of the extracts from *A. lividus*. Gallic acid and BHA were used as reference antioxidants. Values are means ± SD ($n = 3$).

as the positive controls gallic acid (40.3 ± 0.45 μg/ml) and BHA (44.1 ± 3.88 μg/ml) were.

3.5. Hydroxyl radical scavenging activity

Fig. 4 shows that the water, methanol and ethyl acetate extracts from *A. lividus* exhibited dose-dependent inhibition of hydroxyl radicals. At 20 mg/ml, the methanol and ethyl acetate extracts quenched 92.8 ± 0.08% and 91.7 ± 0.78% of the hydroxyl radicals in the reaction mixture, respectively, which was comparable to BHA which quenched 95.2 ± 0.37% at 1.25 mg/ml. However, at 60 mg/ml, the hydroxyl radical scavenging activity of the water extract (60.0 ± 1.83%) was significantly lower ($p < 0.05$) than those observed with the two other extracts and comparable to that of gallic acid (65.4 ± 0.85%) at 20 mg/ml.

With regard to the EC_{50} values, the water extract (37.4 ± 0.43 mg/ml) was a considerably less effective ($p < 0.05$) hydroxyl radical scavenger compared to the ethyl acetate (3.58 ± 0.13 mg/ml) and the methanol (9.53 ± 0.27 mg/ml) extracts. The ethyl acetate and methanol extracts showed less hydroxyl radical scavenging activity than BHA (0.195 ± 0.019 mg/ml), but were much better than gallic acid (13.28 ± 0.11 mg/ml).

3.6. Reducing power

In the reducing power assay, the presence of antioxidants in the extracts results in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. Fig. 5 shows the extent of the reduction, in terms of absorbance values at 700 nm, for the extracts ranging in concentration from 2.5 to 80 mg/ml. The reducing power of the water extract (0.320 ± 0.011) at a dosage of 80 mg/ml, as well as the methanol (0.962 ± 0.0083) and the ethyl acetate (0.561 ± 0.0094) extracts at a dosage of 40 mg/ml, were found to be significantly ($p < 0.05$) below those of gallic acid (1.691 ± 0.0066) at 0.625 mg/ml and BHA (1.695 ± 0.0072) at 2.5 mg/ml. Oboh (2005) reported that *A. cruentus* leaves had the ability to reduce Fe (III) to Fe (II) (1.5 absorbance at 700 nm), whilst it was found in this study that extracts of *A. lividus* had a weaker reducing power.

As can be seen from the EC_{50} values, it was interesting to find that, although the ethyl acetate extract showed the highest antioxidant activity, it was less effective in reducing power (28.19 ± 0.28 mg/ml) compared to that of methanol extract (22.79 ± 0.03 mg/ml). The water extract had the lowest reducing power which was not concomitant with its high antioxidant activity. EC_{50} values of all the extracts in reducing power were significantly different ($p < 0.05$) from the EC_{50} values obtained for gallic acid (0.09 ± 0.002 mg/ml) and BHA (0.053 ± 0.0066 mg/ml). The results were in accordance with Farhoosh, Golmovahhed, and Khodaparast (2007) who reported that the antioxidant activity of different tea extracts may involve other mechanisms in addition to those of reductones.

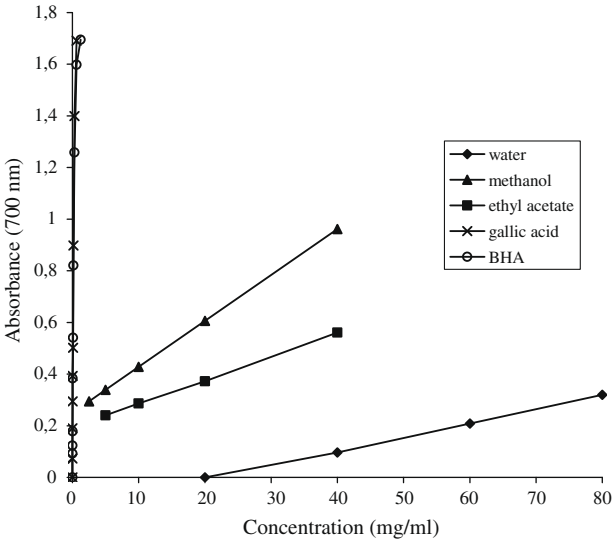

Fig. 5. Reducing power of the extracts from *A. lividus*. Gallic acid and BHA were used as reference antioxidants. Values are means ± SD ($n = 3$).

3.7. Iron chelation (II) activity

By forming a stable iron (II) chelate, an extract with high chelating power reduces the free ferrous ion concentration and thus decreases the extent of the Fenton reaction which is implicated in many diseases (Halliwell and Gutteridge, 1984). All the extracts demonstrated an ability to chelate iron (II) ions in a dose-dependent manner (Fig. 6). Water, methanol, and ethyl acetate extracts chelated ferrous ions by 73.9 ± 1.83% at 40 mg/ml, 87.8% ± 0.12%

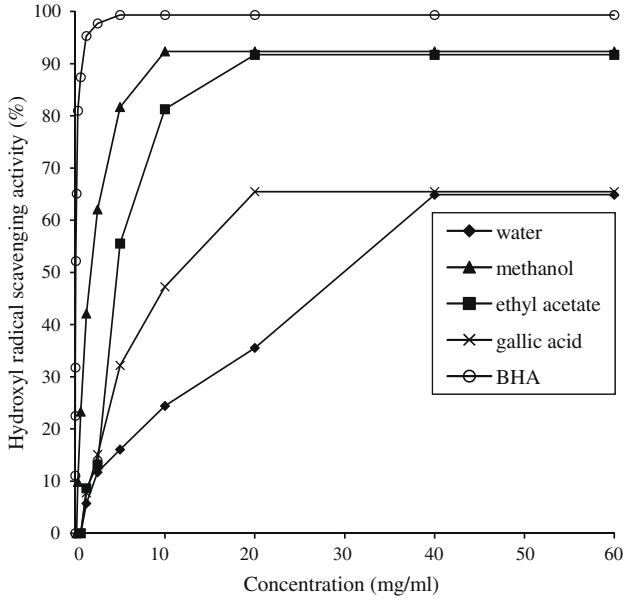

Fig. 4. Hydroxyl radical scavenging activity of the extracts from *A. lividus*. Gallic acid and BHA were used as reference antioxidants. Values are means ± SD ($n = 3$).

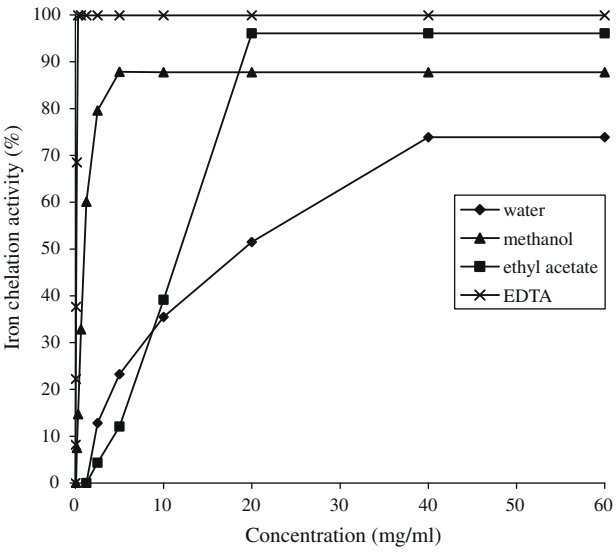

Fig. 6. Iron chelation activity of the extracts from *A. lividus*. EDTA was used as the positive control. Values are means ± SD ($n = 3$).

at 5 mg/ml, and 96.0 ± 0.56% at 20 mg/ml, respectively. EDTA, used as a positive control, showed excellent chelating ability of 99.9 ± 0.43% at a concentration as low as 0.31 mg/ml.

From the estimated EC_{50} values, defined as the concentration of extract required to chelate 50% of the available iron (II), it can be seen that the most effective iron (II) chelating extract was methanol (2.35 ± 0.09 mg/ml), followed by the ethyl acetate (15.6 ± 0.25 mg/ml) and water extracts (27.8 ± 0.57 mg/ml), in decreasing order. These were significantly ($p<0.05$) different in efficacy from the EDTA (0.160 ± 0.003 mg/ml).

The total phenolic contents were correlated with the antioxidant (r^2 = 0.993, 0.892, and 0.973 for water, methanol, and ethyl acetate extracts, respectively) and chelating (r^2 = 0.994 and 0.748 for water and ethyl acetate extracts, respectively) activities of the water, methanol, and ethyl acetate extracts and their scavenging effects on DPPH· (r^2 = 0.992, 0.994, and 0.9999 for water, methanol, and ethyl acetate extracts, respectively), $ABTS^{\cdot+}$ (r^2 = 0.999, 0.997, and 0.775 for water, methanol, and ethyl acetate extracts, respectively) and hydroxyl (r^2 = 0.973, 0.832, and 0.917 for water, methanol, and ethyl acetate extracts, respectively) radicals. These findings seem to suggest phenolics to be important contributors to the antioxidant activity.

The antioxidant activities of water, methanol and ethyl acetate extracts measured in a phosphatidylcholine liposome system were significantly correlated with their scavenging activities on $ABTS^{\cdot+}$ (r^2 = 0.992, 0.961, and 0.990 for water, methanol, and ethyl acetate extracts, respectively), DPPH· (r^2 = 0.971, 0.922, and 0.936 for water, methanol, and ethyl acetate extracts, respectively) and hydroxyl radicals (r^2 = 0.919, 0.869, and 0.965 for water, methanol, and ethyl acetate extracts, respectively), and their iron chelation activities (r^2 = 0.901, 0.708, and 0.941 for water, methanol, and ethyl acetate extracts, respectively), indicating that the antioxidant activities of the extracts may be due to their radical scavenging and chelating activities, and blocking of the chain reaction in the peroxidation of lipids. Therefore, the used methods have satisfactory correlations for the examination of antioxidant activities of the extracts.

Based on the EC_{50} values, the ethyl acetate extract was the most appropriate source of antioxidant compounds.

4. Conclusion

The present study demonstrated that of the water, methanol and ethyl acetate extracts from *A. lividus,* the antioxidant potential was highest in the ethyl acetate extract, followed by methanol extract, whilst the water extract was found to be the least effective antioxidant, which cannot be explained by the total phenolic content. This suggests that the yield and the total phenolic content may not be good indicators of potential antioxidant activity of the extracts. This may be due to the type of phenolics extracted or some unidentified antioxidants. These observations suggest that the nature of the biologically active constituents of the ethyl acetate extract may be different from those present in the methanol and water extracts. As a result, *A. lividus* L. stems with leaves and flowers seem to be good sources of natural antioxidants.

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