Chiang Mai J. Sci. 2011; 38(4) : 560-571
http://it.science.cmu.ac.th/ejournal/
Contributed Paper

Comparative Analysis of Aqueous Extracts of Amaranth and Coriander in Scavenging Free Radical Activity and Protection of DNA against Oxidative Damage

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Received 24 January 2011
Accepted 9 June 2011

ABSTRACT

Green leafy vegetables are **considered to be of immense** therapeutic potential since ancient days. Amaranth (*Amaranthus gangeticus*) and coriander (*Coriandrum sativum*) are green leafy vegetables widely distributed across the world and most of which are consumed as daily diet. Present study was aimed to investigate the antioxidant potential of boiled aqueous extract of leaves of these green vegetables in terms of their total antioxidant capacity, free-radical scavenging using α, α-diphenyl-β-picryl hydrazyl ($DPPH^{\cdot}$) and hematoxilin, anti-lipid peroxidation, metal chelating and ability to protect DNA against oxidative damage. Results of all the *in vitro* free-radical generating assay systems demonstrated positive scavenging efficiency for the extracts of both vegetables. The IC_{50} values for boiled aqueous extract revealed that $DPPH^{\cdot}$ radical scavenging ability were almost same for both the vegetables. The extract showed marked confirmed protective effect on calf thymus genomic DNA against oxidative damage and anti-lipid peroxidation activity against ferrous ions on goat liver homogenates. The extract of amaranth showed better anti-lipid peroxidation activity as compared to coriander extract. The optimum DNA protecting ability against free radical-induced damage was 250 μg and 350 μg for aqueous extracts of amaranth and coriander, respectively. The results, which suggest that amaranth and coriander have nutritive as well as medicinal values against free-radical-associated oxidative damage and related degenerative diseases involving metabolic stress, genotoxicity and cytotoxicity, highlight the importance of undertaking scientific studies of the commonly used vegetables with traditional claims of pharmaceutical significance.

Keywords: antioxidant, DNA damage, free radical scavenger, green leafy vegetables, lipid peroxidation, metal chelating

1. INTRODUCTION

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as

well as infectious diseases. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Plants extract containing especially phenolic and water-soluble antioxidants are widely distributed in plants and presents in fruits, vegetables and beverages of human diet. Consumption of fruit and vegetables rich in flavonoids has been associated with many health benefits [1]. The most common antioxidant compounds present in the fruits and vegetables which provide them their free radical scavenging property are vitamin C, vitamin E, carotenoids, flavonoids, and allied phenolics and thiol compounds [2]. The antioxidant property of polyphenols is a principal feature of their radical scavenging activity. Various phyto-chemicals and their related effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, species, and herbs have been intensively studied [3-6]. Green leafy vegetables are the store-house of useful minerals and vitamins at the cheapest price, commonly available and are considered as the corner stones of health care system in alleviating some serious diseases [7]. Since long, it has been estimated by researchers through epidemiological tests that every *ration* increase in fruit and vegetable consumption reduces the threat of cancer by 15%, cardiovascular disease by 30% and mortality by any cause by 20% [8, 9].

Free radicals and other oxygen derived molecules are produced in the body by leakages in the electron transport chain in the mitochondria and endoplasmic reticulum. If left free they can cause oxidative damage by initiating chain reactions that disrupt membranes, denature proteins, fragment DNA and ultimately participating in the cell death, ageing and cancer [10]. It may cause many health disorders such as atherosclerosis, liver disorder, pesticide toxicity, diabetes mellitus, cancer, neurodegenerative and inflammatory diseases [11]. The diseases developing in our body due to these free radicals can only be terminated if the radicals can be removed from our system by either complete wash-out from the system or by rendering them inactive. This function is performed by special compounds with reducing nature, the antioxidants [12]. The protective role of antioxidant lies in its ability to trap free radicals available in the surrounding environments. They neutralize the free radicals by donating one of their own electrons, ending the electron-stealing reaction. They act as scavengers, helping to prevent cell and tissue damages that could lead to cellular damage and disease. This property of antioxidant has been evidenced by a large number of *in vitro* assays which suggest that compounds like flavonoids have anti-inflamatory, anti-allergic, anti-viral and anti-carcinogenic [6, 13]. Intracellular defense mechanism (e.g., cellular antioxidant enzymes such as catalase, glutathione peroxidase and dietary nonenzymatic antioxidants such as vitamin E, Vitamin C, carotenoides and flavonoids shield cellular macromolecules from this free radical attack. But, when the damage induced by free radicals is more, these intracellular defense mechanisms are incapable of repairing the damage. In such cases, the antioxidants supplements come into play. However, their metal-chelating property cannot be ignored [14]. The plant species contains several thousands of polyphenols but only a limited number of them are important for human health. Since the oxidative DNA damage can play a significant role in mutagenesis, cancer, aging and other human pathologies, the decrease of the oxidative stress seems to be the best strategy possible to achieve by eating food rich in antioxidants and/or by taking supplements containing polyphenols, for example plant extracts [15].

Plant-derived compounds are of interest in this context because they comprise not only antioxidant which save us from free radicals but have safer or more effective substitutes for synthetically produced antimicrobial agents.

Here, amaranth (*Amaranthus gangeticus*) and coriander (*Coriandrum sativum*), green leafy vegetables widely distributed across the world and consumed as daily diet were used for this study. Coriander, also known as cilantro in America, is used as herb as well as spice. Traditionally, it is known for its antidiabetic, anti-inflammatory, cholesterol lowering, carminative, diuretic, stimulant, stomachic, refrigerant, aphrodisiac, analgestic and hypoglycemic properties [16] and also used in anxiety, insomnia, convulsion and dyspeptic complaints [17]. It is rich in antioxidant and known to detoxify metals. *Amaranthus gangeticus* commonly known as amaranth or pigweed are considered as weeds, rich in antioxidant and known for its radioprotective role [18]. Globally, amaranth is used as leafy vegetables, cereals and ornamentals. These plants are reported to contain high amount of nutrients along with other essential bioactive compounds. The aim of this study was to explore these plants in investigating the free radical scavenging and metal chelating activities of the boiled aqueous extract in relation to their antioxidants content.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

The green leafy vegetables *Amaranthus gangeticus* (AG) and *Coriandrum sativum* (CS) were sourced from field and local market of Mednipur district of West Bengal, characterized and identified by experts of Botany Department, University of Calcutta, India.

All chemicals used were of analytical grade purchased from Sigma, Merck and Fluka.

2.2 Preparation of Plant Extract

The extract of AG and CS were prepared according to Rai *et al.,* [19] with some modification. Briefly, 100 ml of water was added to 10 g of each fresh vegetable leaves and concentrated by boiling to one-tenth of its value. After boiling, each of the mixture was filtered using Whatman no. 1 filter paper to obtain a clear solution designated as boiled aqueous extract. The crude extracts were concentrated in a rotary evaporator and the stocks were stored at -20^{o}C for further analysis of their properties under study.

2.3 Determination of Moisture Content

The moisture content of the green leafy vegetables was evaluated by measuring the weight of the leaf sample before and after the water removal by evaporation through convection moisture analyzer (Kern-MLS). Moisture percentage in the sample was calculated using the formula,

Moisture % = $W_1 - W_2 / W_1 \times 100$

where, W_1= Initial Sample Weight

and W_2= Final Sample Weight

2.4 Determination of Total Protein, Phenol, Flavonoids and Antioxidants Capacity

The plant extracts were tested for their total protein content to compare their nutritive value. This was carried out in accordance with the procedure by Lowry method [20]. The phenol content of the plant extract was determined by Folin-Ciocalteau method [21] with minor modification. To 1 ml of water, 100 µl of each of aqueous extract was added. Then 0.5 ml of Folin Ciocalteu reagent was added to each set. The mixture was vigorously shaken and was incubated for 3 min at room temperature. After the incubation, 1 ml of 20% Na_2CO_3 was added. The mixture was heated for 1 min in an incubator at 95^{o}C and

the absorbance of the developed colour was recorded at 765 nm using an UV-VIS spectrophotometer and was expressed as Gallic acid equivalents (GAE) per gm of plant weight.

The flavonoids content was observed qualitatively following Ebrahimzadeh1 [22] with minor modification. In brief, the extract of the plant was mixed with solution of hydrated aluminum chloride. The resultant solution obtained was incubated at room temperature for 30 minutes. The absorbance was noted at 440nm.

For determination of total antioxidant activity, the assay was based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green solution [23]. Briefly, an aliquot of 0.1 ml boiled aqueous extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. On completion of incubation period samples were cooled to room temperature (27°C), the absorbance was measured at 695 nm against blank. A typical blank contained 1 ml of reagent solution along with appropriate volume of water and was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities were expressed as equivalents of ascorbic acid (Ascorbic acid equivalents mg/g).

2.5 Antioxidant Evaluation Assays

The antioxidant activity of the aqueous extract of the two leafy vegetables under study was evaluated by employing the following methods:

2.5.1 Radical scavenging activity using DPPH· and superoxide radical by Hematoxilin

The antioxidant activity of the extract was measured on the basis of the scavenging activity of the stable DPPH· free radical [24] with minor modification. In brief, boiled aqueous extract was taken in total volume of 1 ml and to each of the set 4 ml of 0.1 mM methanolic DPPH· solution was added. The reaction mixture was mixed well and allowed to stand for 30 min at room temperature. The change in absorbance of the samples was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage calculated using the following formula,

Percentage inhibition activity

$$= [(A-B)/A)] \times 100$$

where, A = absorbance of the blank solution and B = absorbance of the test solution.

Superoxide radical (O_2^-) was generated from auto oxidation of hematoxilin and was detected by an increase in absorbance at 560 nm, in a spectrophotometer (19). The reaction mixture contained 0.1 M of phosphate buffer (pH 7.4), EDTA (0.1 M), hematoxilin (50 μM) and incubated at 25°C for different time periods. Inhibition of auto oxidation of hematoxilin by boiled aqueous extracts over the control were measured using the following formula,

Percentage inhibition activity

$$= [(A-B)/A)] \times 100$$

where, A = absorbance of the blank solution and B = absorbance of the test solution.

2.5.2 Anti-lipid peroxidation effect of aqueous extract

A modified thiobarbituric acid-reactive species (TBARS) assay [25] was done to measure anti-lipid effect of the green leafy vegetables aqueous extracts. Goat liver homogenates (10% w/v) were prepared in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 8,000 rpm for 10 min. The supernatant was used for the study lipid peroxidation. Different concentrations of boiled aqueous extract of

amaranth and coriander were taken in screw capped tubes. Liver homogenate (0.5 ml) were added to all the test tubes. Total volume was adjusted to 1 ml by double distilled water. $FeSO_4$ (0.07 M) was added to each test tubes for initiation of peroxidation and incubated for 30 min at 37°C. Then 20 % acetic acid (1.5 ml), 0.8 % TBA in 1.1 % SDS (1.5 ml) and ice chilled 20 % TCA (50 µl) were added to all the test tubes and were gently vortexed. The reaction mixtures were heated at 95°C for 1 h. After completion of incubation, samples were cooled, 5 ml of butan-1-ol were added to each tube and centrifuged at 300 rpm for 10 min. Absorbance of the organic layer was measured at 532 nm. An identical experiment was performed in the absence of aqueous extract as control. Anti-lipid peroxidation (ALPO) was calculated by using the formula,

ALPO % = 1- (E/C) × 100

where, E was absorbance of sample and C was absorbance of blank

2.5.3 Effect of aqueous extract on the in-vitro oxidative damage of genomic DNA

The procedure described by Karia *et al.,* [26] was followed with minor modifications. 50 µg of Calf Thymus (CT) DNA, 50 µM Mohr's salt (Fe^{2+} ions) as the oxidizing agent and varying amount of plant extract were added in a total volume of 25 µl and then incubated at 37°C for 30 min. Control set contain only CT DNA and 50 µM of Mohr's salt under similar condition. After oxidative treatment, the DNA was immediately subjected to electrophoresis in 0.9 % agarose gel, in 1x TAE buffer (40mM-Trise-acatate, 1mM EDTA), followed by ethidium bromide staining (50 µg/ml) and visualized by gel documentation system. The resulting fragmentation and inhibition of fragmentation were analyzed.

2.5.4 Copper (Cu^{2+}) ions chelating activity

UV-Visible spectra were analyzed by the method of *Jonathan et al.,* [14] with minor modifications. Briefly, 10 mM PBS (pH 7.4) containing 100 µg/ml plant extracts were kept at room temperature, and the absorption spectra were recorded between 200 and 500 nm. A number of scans with 25, 50, 75 or 100 µM $CuSO_4$ were taken after 10 min and compared with extract alone. Then the effect of EDTA (100 µM) was tested for the extract supplemented with Cu^{2+} complex. All spectra were run against blanks containing the PBS buffer and $CuSO_4$.

2.6 Statistical Analysis

All experiments were repeated at least three times. The IC_{50} values (concentration of sample for 50% inhibition) were also calculated for the aqueous extract. Results were reported as Mean ± SD. Mean data of the results obtained was analyzed using ANOVA and subjected to least significant difference (LSD) for studied parameters comparisons.

3. RESULTS AND DISCUSSION

3.1 Total Protein, Phenol, Flavonoids and Antioxidants Capacity

Results of the biochemical assays carried out with the boiled aqueous extract of *A. gangeticus* (AG) and *C. sativum* (CS) revealed some variation between the two leafy vegetables (Table 1). The moisture content of the green leafy vegetables was 86.78 % for CS and 86.50 % for AG. Both the extracts were rich in protein, total antioxidant capacity as well as associated with some amount of phenol content. Result showed that extracts of CS had a significantly higher ($p< 0.05$) total protein content (29.0 mg/g) than the extract of AG (13.8 mg/g). Presence of flavonoids was also detected in both leafy vegetables though could not be quantitatively estimated. Flavonoids have been reported to contain

antioxidant and free radical scavenging activities in vegetables as well as the ability to protect membrane lipids from oxidation [27]. AG was found to be more enriched in terms of the total antioxidant activity and phenol content (14.1 mg/g and 0.43 mg GAE/g, respectively) as compared to CS (12.1 mg/g and 0.15 mg GAE/g respectively). Total antioxidant activity of AG had a significantly higher ($p< 0.05$) value than the extract of CS extract. Phenols were crucial plant antioxidants that scavenge free radicals and played a pivotal role in stabilizing lipid oxidation. Previous reports for human indicated that consumption of 1 g of polyphenolic compounds from the diet rich in fruits and vegetables can inhibit the chances of mutagenesis and carcinogenesis [28]. The total phenol content was low for both vegetables suggesting that the aqueous extract may limit the extraction and solubilization procedure of phenols. Although many important bioactive compounds are still being present in both the extracts. These components are apparently responsible for their high antioxidant capacity and thus protective ability against free radical cellular damage.

3.2 Antioxidant Testing Assays

3.2.1 Radical scavenging activity using DPPH· and hematoxilin assays

The free radical scavenging capacity of plant bioactive components is a basis of the high antioxidant activity of herbal medicines [29]. DPPH· is a stable free radical which accepts an electron or H radical to become a stable diamagnetic molecule. The technique involved reaction of definite compounds or extracts with DPPH· in methanol solution. This type of reaction has been widely used to test the capability of the compound or extracts to operate as free radical scavengers [30]. Reduction of the DPPH· radicals can be observed by the decrease in absorbance at 517 nm. The data presented in Table 1 showed boiled aqueous extract of both AG and CS had good DPPH· radicals scavenging activity. The IC_{50} value, a measure of the extract concentration that was required for 50% inhibition of the free radical DPPH·, was determined. The IC_{50} values for extract of studied vegetables revealed that DPPH· radicals scavenging ability were almost same for both AG and CG i.e., non-significant (Table 1). Boiled aqueous extract of a few Malaysian vegetables have been reported to contain high amount of antioxidant and DPPH· generated free radicals scavenging activities [31]. Similar reports on free radical scavenging activity in various green leafy vegetables have been reported from other sources also. The increased production of free radical appeared to be a trait of most, if not all human diseases, including cardiovascular disease and cancer. Therefore, such dietary

Table 1. Total protein (TP in mg/g), total phenol content (TPC in GAE equivalents mg/g), total antioxidant activity (TAA in Ascorbic acid equivalents mg/g) in mg/g and LC_{50} in mg/ml for radical scavenging and anti-lipid peroxidation (ALPO) of amaranth and coriander boiled aqueous extract (values are mean ± SD of three determinations). Means with different letters in the same column differ significantly (LSD test, $P < 0.05$).

Sample	TP	TPC	TAA	IC_{50} (mg/ml)		
				DPPH·	Hematoxilin	ALPO %
Amaranth	13.8 ± 0.4[a]	0.43 ± 0.05[a]	14.1 ± 0.4[a]	0.54 ± 0.7[a]	0.36 ± 0.04[a]	1.5 ± 0.2[a]
Coriander	29.0 ± 0.5[b]	0.15 ± 0.08[a]	12.1 ± 0.6[b]	0.53 ± 0.2[a]	0.48 ± 0.07[a]	1.8 ± 0.7[a]

antioxidants from such vegetables may be particularly important in fighting these diseases by conferring protection against free radical damage to cellular biomolecules like DNA, proteins and lipids. A variety of biological and photochemical reactions generate superoxide radical ($O^{\bullet}_{2}^{-}$) which is a highly toxic species. Hematoxilin auto-oxidation inhibition was observed by the aqueous extract of AG and CS. The aqueous extract showed both vegetables contained water-soluble superoxide radical scavenger although IC_{50} of CS was higher than AG (Table 1). So, here, amaranth extract was a better superoxide radical inhibitor as compared to coriander.

3.2.2 Effect of aqueous extract on the in-vitro anti-lipid peroxidation

Free radical induced lipid peroxidation has gained vast importance, because of its association in the etiology of a number of pathophysicalogical conditions. The anti-lipid peroxidation activity on goat liver homogenates of the aqueous extract in presence of ferrous sulphate is shown in Table 1. The extract of amaranth showed better anti-lipid peroxidation activity as compared to coriander extract. Fruits and vegetables contain vitamin C, gallic acid, tannins, anthocyanins and many important bioactive components apart from their nutritive components. Tannins are a class of water soluble polyphenols present in many vegetables. The phenolic content of amaranthus aqueous extract is higher than coriander accounting for its higher anti-lipid peroxidation activity. Polyphenols vegetables juices are major contributors of antioxidant activity because of their hydrogen donor property [25].

3.2.3 Influence of aqueous extract on the oxidative damage of genomic DNA by Fe^{2+} ions

The ability of various plant extracts to prevent DNA damage or to stimulate enhancement of DNA repair pathways are being widely studied by researchers from time to time. Improvement of DNA repair activity due to stimulation of repair enzymes by lymphokinases or cytokines in lymphocytes has been reported with *Iscader*, a water extract from *Mistletoe* [32]. Enchanced DNA repair has also been shown with C-MED-100TM, an aqueous extract from *Uncaria tomentosa* [33] and extracts of *Brussels* sprouts [34]. *Amaranthus paniculatus*, a common weed often taken as vegetables by the rural population, has been successfully tested against gamma radiation [35]. Plants like sugarcane have already been shown to possess antioxidant activity and exhibit protective role against radiation induced DNA damage [10]. In our study oxidative damage of CT DNA by optimum amount of Fe^{2+} ions (50 μM) followed by treatment with varying amount of boiled aqueous extract of each vegetables (ranging from 100 μg to 500 μg) after incubation for 30 min at 37°C was studied through agarose gel electrophoresis. As visualized by agarose gel electrophoresis, AG and CS extract conferred a dose-dependent DNA protection against oxidative damage induced by Fe^{2+} ions.

The untreated sample DNA was compact with a high molecular weight showing its integrity as indicated by agarose gel electrophoresis (Figure 1, lane 1). Fe^{2+} ions treated CT DNA gets fragmented as evident from the smear nature of the DNA band (Figure 1, lane 2), On the other hand, addition of GLV water extract, produces the opposite effect. The DNA cleavage induced by the free radicals generated by Fe^{2+}extract gets reverted restoring the DNA to its initial tight, compact, native state. The optimum concentrations of amaranth extract were 250 μg and coriander extract 350 μg in offering maximum DNA protecting ability (Figure 1, lane 3 and 4). From

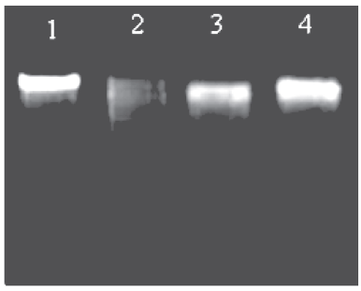

Figure 1. Effects of plants extract on Fe^{2+} ions stressed DNA as monitored by electrophoretic mobility. 50 μg/ml of CT DNA was oxidized by 50 μM Mohr's salt (Fe^{2+} ions) in presence of optimized amount of aqueous extracts, for amaranth and coriander, respectively for 30 min at 37°C. DNA profiles was visualized by electrophoresis on 0.9 % agarose gel and stained with ethidium bromide (50 μg/ml). Lane 1, CT DNA, 2, CT DNA + Fe^{2+}, lane 3-4, CT DNA + Fe^{2+} + aqueous extracts (Amaranth and Coriander)

the result it is tempting to speculate that green leafy vegetables plays a protective role against oxidative damage of DNA simply by scavenging Fe^{2+} ions generated free radicals.

3.2.4 Copper (Cu^{2+}) ions chelating activity

Metal chelating activity of the plant extracts were tested by monitoring the change of the UV-Visible absorption spectra of aqueous extract upon addition of Cu^{2+} ions at pH 7.4. The effect of Cu^{2+} on the spectral characteristics of each extract is represented in terms of shifting nature of bands in both amaranth and coriander extract (Figure 2 a and b).

The change in spectral nature of AG is attributed to the formation of a chelate complex of boiled aqueous extracts with Cu^{2+} ions. Interactions of Cu^{2+} ions (100 μM) with extracts produced bathochromic shift in band I (327 nm) with decrease in absorbance. Band II (220 nm) demonstrated a small bathochromic shift (2 nm) and was accompanied by slight increase in absorbance (Figure 2a, dotted line). Isosbestic point at 344 nm and 281 nm suggests the direct transition from one form (extract) to another form (Extract- Cu^{2+} ions chelate). The formation of amaranth extract - Cu^{2+} ions chelate was confirmed with reaction of EDTA, a well known chelating agent for metal ions. When 100 μM of EDTA was added to the **Cu^{2+}** ions chelated extract-, the shifted bands returned back to their initial position with decrease in absorbance (Figure 2a, thin solid line).

Under similar conditions, band I of the CS extract demonstrated a 35 nm bathochromic shift in the presence of 100 μM Cu^{2+} ions, which was associated with decrease in the absorbance. No changes in the position of band II were seen. Isosbestic point at 344 nm and 277 nm suggests the direct transition from one form (only extract) to another form (Extract- Cu^{2+} ions chelate). On adding EDTA (100 μM), the original spectrum was recovered (Figure 2B).

The spectroscopic studies showed that green leafy vegetables are capable of chelating metal ions namely the Cu^{2+} ions. Both extracts had shown good metal chelating activities, and hence can serve as potential reducing agent. Flavonoids are known for their reducing properties and chelating action for metal ions (Cu^{2+}) [14]. The chelating agents may inhibit lipid oxidation by stabilization of transition

metals. Reports are there with compounds bearing ortho-dihydroxyl groups having roles in chelating transition metal ions [36]. Our study again re-established apparently that coriander & amaranth extracts promoted the formation of metal complex.

It has been reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilising the oxidized form of the metal ion. Earlier results conducted upon the aerial part extract of *Ornithogalum sintenisii* showed higher Fe^{2+} - chelating activity than the bulbs but it was not as strong as that of EDTA. The higher

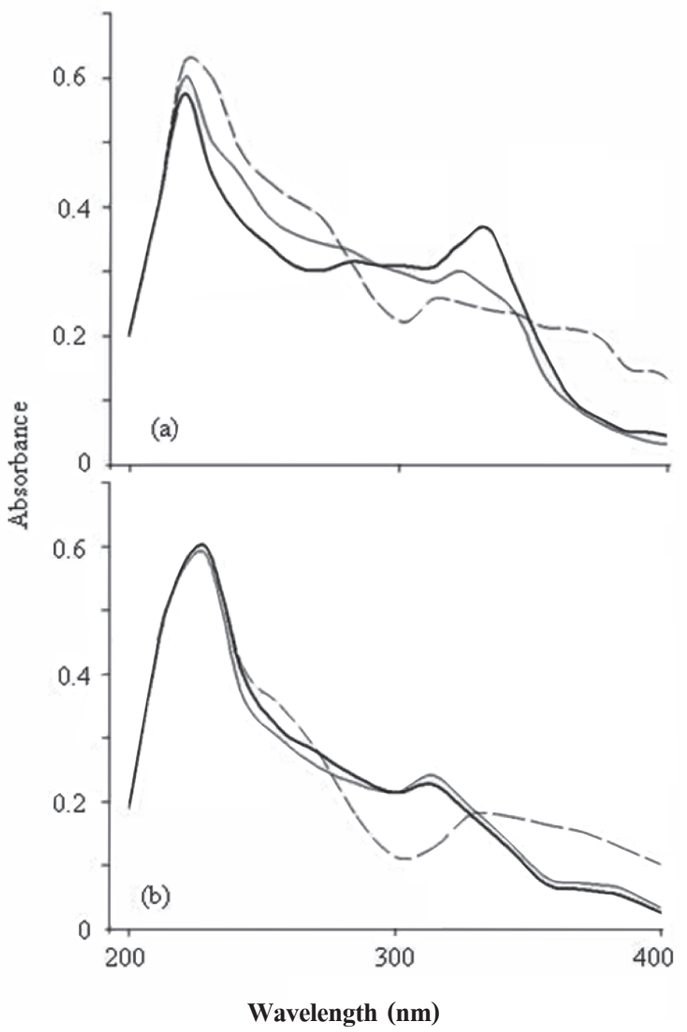

Figure 2. Effect of Cu (II) & EDTA on absorption spectra of plant water extract (a, Amaranth and b, Coriander). Extracts (100 μg/ml) and 100 μM Cu (II) incubated for 10 min. After this EDTA (100 μM) was added. Thick solid line, extract (100 μg/ml); dotted line, extract (100 μg/ml) + Cu^{2+} (100 μM); thin solid line, extract (100 μg/ml) + Cu^{2+} + EDTA. All the spectra were recorded in 10mM PBS (pH 7.4).

total phenol and flavonoid contents of the aerial parts might have led to the higher reductive potential of the extract [22]. So in this context, our experimental result is a promising one to carry out further studies with purified bioactive compound present in the boiled aqueous extract of AG and CS.

4. CONCLUSIONS

Generally, green leafy vegetables are consumed either in the cooked form or served as salads. It can be seen that these vegetables exhibited antioxidant activities at varied levels depending upon the cultivar of plants in different systems. Keeping all the results in mind, it can be inferred that both AG and CS are suited for remedial purposes. AG and CS boiled aqueous extract have high antioxidant activity as revealed by their scavenging nature of free radicals. Both green leafy vegetables had shown good result in terms of metal chelating activity and DNA protection role. The estimation of endogenous substances with biological antioxidant role is of great importance and may lead us in assisting future drug design. If analysis of studied extracts can be performed in depth it may bear assurance for cures of many deadly diseases caused by pathogens and mutation due to free radicals. Being non-synthetic they are free from side effects causing minimum damage to the endogenous system compared to that of synthetic drugs.

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