STM JOURNALS

Research & Reviews: A Journal of Biotechnology
ISSN: 2231-3826 (Online), ISSN: 2347-7245 (Print)
Volume 8, Issue 1
www.stmjournals.com

Biochemical Assays of Crude and Purified Oxycarotenoid Extracts Isolated from Coriander Leaves and their Effect on Oxidative Stability of Oils by Rancimat Assay

Sherena P.A.[1,*], Annamala P.T.[2], Mukkadan J.K.[1], Dinesha Ramadas[3]

[1]Department of Biochemistry, Little Flower Medical Research Center, Angamaly, Kerala, India
[2]Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, India
[3]Adichunchanagiri Institute for Molecular Medicine, AIMS – Central Research Laboratory, B.G. Nagara, Karnataka, India

Abstract

Antioxidants are important substances in the fight against the damage done by free radicals that are produced by oxidative stress. Antioxidants are available in various forms as dietary supplements/synthetic forms. Because of their toxicity and side effects, the extensive use of many of these synthetic antioxidants is ruled out. Most nutritionist agrees that, the natural food is the best source of antioxidants. Dietary antioxidants have the ability to scavenge free radicals, acts as chain inhibitors and metal chelators and thereby prevent or retard chronic health diseases. In the present study, coriander leaves were selected as the dietary source because of carotenoid content and described their extraction, saponification and isolation process of oxycarotenoid concentrate. The assessment of both crude extract and isolated oxycarotenoid extract was also described for its effective antioxidant activity by different model systems such as hydroxyl radical, DPPH, Ferric ion reducing activity, Ferrous ion chelating activity, Lipid peroxidation and SOD activity. Oxidative stability of oils was also assessed by Rancimat study. The results showed that the crude and purified extracts of coriander leaves could act as better antioxidant to prevent the toxicity and also proved that the purified extract showed more antioxidant activity when compared to the crude extract. The estimated IC_{50} values of purified extracts in model systems such as DPPH, hydroxyl radical and superoxide activity were 14.06 μg/ml, 39.79 μg/ml, 37.57 μg/ml, respectively. It was found to be significantly inhibited the lipid peroxidation by $FeSO_4$–Ascorbic acid system with IC_{50} values at 71.4 μg/ml. The oxidative stability of oils was increased from 4.31 h to 6.05 h and 4.31 h to 7.03 h by the addition of crude and purified extracts of coriander leaves, respectively. These results showed that that coriander leaves could be used as the dietary source of antioxidants.

Keywords: *coriander leaves, oxycarotenoid, antioxidant, flash chromatography, rancimat*

****Author for Correspondence*** E-mail: sherenapa@gmail.com

INTRODUCTION

Plants are well known to produce a diverse array of secondary metabolites such as alkaloids, terpenes and phenols to engage with the world around them. Phenols like polyphenols play an important role in plant protection against UV radiation, pathogens and predators, the plant growth and reproduction. The quantity and type of polyphenols vary from species and zones [1, 2].

There are numerous evidences that, a diet rich with plant foods with large amounts of antioxidants such as Vitamin C, E or natural antioxidants such as flavonoids, tannins, polyphenols, carotenoids and terpenoids can prevent oxidative stress and chronic degenerative human aliments [3]. Oxidative stress and inflammation reactions are due to the free radical formation that have been identified [4]. Free radicals are generated in the body as a byproduct of normal metabolic process and also by external causes such as stimulation, infections etc [5]. Dietary antioxidants are protective compounds which have the ability to scavenge free radicals,

radical chain inhibitors, metal chelators and thereby retard or prevent such diseases [6].

Synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA), Butylated Hydroxy Toulene (BHT) also appear to be promising but it was also reported that, at a dose more than 400μM causes stomach tumour in mice models and hence, it was ruled out for extensive use. Many natural antioxidants derived from plants play an important role because of their safe use with no side effects. Flavonoids and carotenoids are the two well known antioxidants. Carotenoid is one such multidimensional lipid soluble antioxidant present in plants especially in green vegetables. Plant sources such as green vegetables, fruits can be used to derive maximum health benefits because of their carotenoid contents. An important and interesting medicinal plant is coriander (*Coriandrum sativum* L.), a member of the Apiaceae family. In Indian traditional medicine, coriander is used in the disorders of digestive, respiratory and urinary systems as it has diaphoretic, diuretic, carminative and stimulant activities [7]. Coriander leaves are also rich in phytochemicals such as polyphenols, carotenoids and essential oil such as linalool, which shows higher free radical scavenging activity.

Herein, we choose coriander leaves as the present study aimed to extract and isolate oxycarotenoids. The extraction process was followed by solvent extraction, saponification and finally extracted oxycarotenoid using flash chromatographic techniques. The crude and purified oxycarotenoid extract was carried out for *in vitro* biochemical assays. Also the study aimed to assess the effect of both extracts on oxidative stability of oil by rancimat assay. The results of the present study showed that coriander is one of the dietary sources of carotenoids and the carotenoid rich portion scavenges the free radicals effectively.

MATERIALS AND METHODS

Plant Material

Coriander leaves were purchased from the market. Leafy materials were separately washed with tapwater followed by 0.9% salt water and 0.1% $KMnO_4$ solution to make them free from pesticides and insecticides. The leaves were dried at ambient temperature. The extraction and analysis were carried out each time freshly.

Solvents

Acetone, Hexane, Ethylacetate, Methanol, Ethanol, THF were of AR grade, purchased from Merck, India.

Reagents

Potassium hydroxide, Vitamin E acetate, 1,1-diphenyl-2-picryl hydrazyl, Sodium nitroprusside, Sulfanilic acid, Ethylene diamine tetra acetic acid, Ferric chloride, Ferrous sulphate, Nitro blue tetrazolium, Potassium ferricyanide, Trichloroacetic acid, Hydrogenated vegetable oil and natural tocopherol from E Merck and reputed chemical house. Silica gel of mesh size 100–200 was also purchased from Merck. All other reagents of analytical grade were purchased from the Merck Co., India and S.D. fine chem., India.

METHODS

Preparation of Crude Extract of Coriander Leaves

The dried leaf was flaked using a flaker and was extracted with acetone (1:10 w/v) at 40 °C for 2 h under soaking and circulation. The miscella was drained out and the extraction was repeated four times more with acetone at 40 °C. All miscella were collected and filtered through Whatman No.1 filter paper. Distill off the solvent at controlled temperature of about 50–60 °C using rotary evaporator and at the final stage mild vacuum was applied to remove the residual solvents. The concentrate obtained was analyzed, studied for biochemical assays and further processed for the isolation of carotenoids enriched fractions.

Isolation of Carotenoid from the Extract

The concentrate was saponified by dissolving the residue in ethanol (1:2 w/v) and mixed with potassium hydroxide solution (30% w/w) for a period of 3–5 h at 85–90 °C. After the complete saponification, ethanol was removed from the mixture. Then the mixture was diluted with water followed by liquid–liquid extraction using ethyl acetate as solvent. The extraction was repeated till the resultant extract was colourless. The ethyl acetate layer

was collected and concentrated to obtain the saponified concentrate which contains carotenoid was confirmed by high-performance liquid chromatography (HPLC). The saponified extract was subjected to flash chromatography for the separation of carotenoids.

Flash Chromatography

Instrumentation

Carotenoids such as β-carotene and oxycarotenoid such as cryptoxanthin, lutein and zeaxanthin were separated from the saponified crude extract by using Yamazen's Smart Flash Akros chromatography. The instrument is equipped with vacuum pump with controller model No.580S, sample injection system, plastic columns and precolumns, TLC image reader, fraction collectors and detectors. It is a simple, fast and economic approach to preparative liquid chromatography for purification of chemical species.

Silica gel of mesh size 100–200 was used as an adsorbant and packed in the selected column of medium size (L). The saponified crude extract was dissolved in hexane and loaded on to the top of the column filled with silica gel. The flow rate was 5 ml per minute and the compounds were eluted for 45 min successively with hexane, hexane/acetone 95/5; hexane/acetone 80/20 and with acetone. The eluted fractions were collected separately and evaporated to dryness and residue was monitored by HPLC for the confirmation of xanthophylls.

BIOCHEMICAL ASSAYS

The crude extract and the oxycarotenoid concentrate isolated from coriander leaves was used for the assessment of its antioxidant activity using hydroxyl radical, DPPH, ferric ion reducing activity, ferrous ion chelating activity, lipid peroxidation and SOD activity model systems.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of extracts was done according to the method of Halliwell and Gutteridge [8] with minor modifications. The reaction mixture contained in a final volume of 1 ml as $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM) and 2-deoxy-D-ribose (2.8 mM), potassium phosphate buffer (20 mM pH 7.4) and were mixed with or without extract of coriander leaves at various concentrations and incubated for one hour at 37 °C. The mixture was heated at 95 °C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm using the negative control without any antioxidant was considered 100% oxidation. The percentage hydroxyl radical scavenging activity of coriander was determined.

Diphenyl-2-picrylhyrazyl Scavenging Assay

DPPH is a stable purple coloured nitrogen-centered free radical that gets reduced to a yellow coloured diphenylpicryl hydrazine by the fractions in a concentration-dependant manner. DPPH radical scavenging activity was assessed according to the method described by Aquino *et al.* [9]. About 10 μM concentration of coriander leaves extracts was mixed with 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer. The resulting reaction mixtures were incubated at 37 °C for 30 min, and the absorbance was measured at 517 nm.

Lipid Peroxidation Inhibition Activity

The evaluation of antioxidant activity of extracts of coriander leaves at various concentrations based on the inhibition of peroxidation in linoleic acid micelles was done according to Shimazaki *et al.* [10]. The evaluation of oxidation was done by measuring the TBA reactive substances [11]. About 100 μl of linoleic acid was subjected to peroxidation by ferrous sulphate and ascorbic acid (10:100 μmol) in final volume of 1 ml of Tris buffered saline (20 mM, pH 7.4,150 mM NaCl) and various concentrations of coriander leaves. The contents were incubated for 1 h at 37 °C. The reaction was terminated by the addition of 10 μl of 5% phenol and 1 ml of 1% TCA. To each system 1 ml of 1% TBA was added, the contents were kept in a boiling water bath for 15 min, cooled and centrifuged at 6000 rpm for 10 min. The absorbance of

supernatants was measured spectrophotometrically at 535 nm. Appropriate blanks were included for each measurement. The negative control without any test sample was considered as 100% peroxidation. The % inhibition of lipid peroxidation was determined accordingly by comparing the absorbance of the test samples with negative control.

Superoxide Radical Scavenging Activity

The superoxide radical ($O_2^{\bullet-}$) scavenging activity of extracts were measured according to the method of Lee *et al.* [12] with minor modifications. The reaction mixture containing 100 µl of 30 mM EDTA (pH 7.4), 10 µl of 30 mM hypoxanthine in 50 mM NaOH, and 200 µl of 1.42 mM nitro blue tetrazolium with or without extracts and SOD serving as positive control at various concentrations ranging from 0 to 50 µl. After the solution was preincubated at ambient temperature for 3 min, 100 µl of xanthine oxidase solution (0.5 U/ml) was added to the mixture and incubated for one hour at 37 °C, and the volume was made up to 3 ml with 20 mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min and absorbance was measured at 560 nm. Appropriate controls were included to rule out the artifacts induced reaction. The control was without any inhibitor. Inhibitory effect of extracts on superoxide radicals were calculated.

Ferric Ion Reducing Power (Fe^{3+} to Fe^{2+})

The ferric ion reducing power activity of extracts was determined according to the method of Wang *et al.* [13]. About 100 µl of potassium ferricyanide solution (4 mM) was mixed with 200 µl of 20 mM phosphate buffer pH 6.5 in the presence or absence of crude and purified extracts of coriander leaves at various concentrations ranging from 0 to 80 µg. The contents were incubated at 50 °C for 20 min. About 200 µl of 10% trichloroacetic acid was added to the reaction mixture and centrifuged at 5000 rpm. The resulting supernatant was taken and mixed with 100 µl of ferric chloride ($FeCl_3$) solution (2 mM) and final volume was made up to 1 ml with water and then incubated at 37 °C for 10 min. The absorbance was recorded at 700 nm in spectrophotometer. Absorbance increases with increase in reducing power.

Ferrous Ion Chelating Ability (Binds Fe^{2+})

Ferrous ion chelating activity of crude and purified extracts was measured according to the method of Suter and Richter [14]. The reaction solution containing ferrous chloride ($FeCl_2$ 200 µM) and potassium ferricyanide (400 µM) with or without extracts or EDTA at various concentrations ranging from 0–100 g were added in final volume of 1 ml water and mixed. The reaction mixture was incubated at 20 °C for 10 min. Formation of potassium hexacyanoferrate complex was measured at 700 nm in Shimadzu spectrophotometer. The assay was carried out at 20 °C to prevent Fe^{2+} oxidation. Lower absorbance indicated higher iron chelating capacity. The control was without any chelating compound or test sample. The percent ferrous ion chelating effect was calculated.

OXIDATION STABILITY OF OIL

Rancimat Method

Instrumentation

The oxidative stability of oil was evaluated by measuring the length of the induction time determined by Rancimat tests. Evaluations were performed with a 743 Rancimat (Metrohm, Switzerland) and were equipped with two independent heating blocks that allow up to eight samples to be analyzed. Sample weighed out in glass beakers covered with inbuilt conductivity cell. All functions of the 743 Rancimat were controlled by the Rancimat StabNet software.

During the measurement, a stream of air is passed through the oil or fat sample contained in a sealed and heated reaction vessel. This treatment results in oxidation of oil or fat molecules in the sample, with peroxides initially being formed as the primary oxidation products. After some time the fatty acids are completely destroyed; the secondary oxidation products formed include low-molecular organic acids in addition to other volatile organic compounds. These are transported in the stream of air to a second vessel containing distilled water. The conductivity in this vessel is recorded continuously. The organic acids can be detected by the increase in conductivity. The time that elapses until these secondary reaction products appear is known as the induction time, induction period or Oil Stability Index (OSI) [15].

Crude and purified extracts of coriander were mixed in vegetable oil are weighed out the vessel and air supplied into the vessel at 20 ml/min. Under these conditions the lipoperoxidative process reaches its final steps and the short chain volatile acids produced are recovered and measured conductometrically in distilled water. The time required to produce a sudden increase in conductivity determines an induction time which is a measure of the stability of oil.

RESULTS AND DICUSSIONS

An effort to develop an economic process for the preparation of crude and purified extracts from coriander leaves containing carotenoid was done as explained. The crude extract from the grinded leaves were obtained by the solvent extraction followed by concentration. Here the solvent acetone was used in the extraction step, resulting in effective solubilization of material in the solvent and thereby efficient separation of crude extracts from the matrix. The saponification of crude extract was carried out using alcohol and alkali followed by distillation and liquid–liquid extraction. This results in liberation of xanthophylls in free form along with alkali salts of fatty acids such as palmitic, myristic, stearic acid, etc. The obtained concentrate was analyzed by HPLC (Fig. 1). Subjecting the saponified concentrate to flash column chromatography using a column of silica gel and elution using nonpolar solvent to remove beta-carotene and further eluting the column with a mixture of nonpolar/polar solvents in different concentrations and therby obtaining the purified concentrate carotenoid isolated from coriander leaves was confirmed by HPLC analysis (Fig. 2).

The summarised results in Table 1 indicate clearly that purified extract exhibits significant antioxidant activity in terms of its free radical scavenging activities than crude extracts. Lesser the IC_{50}, better is the antioxidant and free radical scavenging activity. Results are expressed as mean± SD; n=3. The iron chelating activity does not near 50% and hence its IC50 cannot be calculated.

Figure 3 shows that purified extracts isolated from coriander leaves more effectively scavenged DPPH radicals in a concentration-dependent manner with IC_{50} value of 14.06 µg and the crude extracts showed IC_{50} value as 17.06 µg. The radical scavenging activity may be due to hydrophobic nature and the presence of conjugated double bonds.

Dose-dependent hydroxyl radical scavenging activities of crude and purified extracts were studied. The efficacy of crude and purified extracts was found to be in a dose-dependent manner. As shown in Figure 4, crude extracts such as coriander showed a maximum inhibition of 28.1±2.1% at a dose of 50 µg and the purified extracts coriander showed a maximum inhibition of 58.1±1.2% at a dose of 50 µg. The purified extract scavenged the hydroxyl radical with IC_{50} values of 39.79 µg/ml. Hydroxyl radicals and nitrogen monoxide as well as many related radicals may cause damage to membrane lipids and DNA strand breaks or DNA base of the genetic substance [16]. Thus crude and purified extracts of coriander could be better antioxidant to prevent the toxicity of OH° radical to deoxyribose than that of synthetic scavenger.

Dose-dependent lipid peroxidation inhibition activities of crude and purified extracts were studied with crude and purified extracts taken at a range of 0–50 µl doses. As shown in Figure 5, the crude and purified extract of coriander effectively inhibits ferrous sulphate: ascorbate (a well known inducer of lipid peroxidation) induced linoleic acid membrane lipid peroxidation dose dependently showing a maximum inhibition of 28.1±0.3% and 36.3±0.9% at a dose of 50µl respectively. Capability of lipid peroxidation by purified extracts was dose dependant and shown IC_{50} alue at 71.4µg/ml. Thus crude and purified extracts of coriander showed antioxidant activities and hence prevent formation of lipid peroxidation.

Superoxide anions produce other kinds of cell damaging free radicals and oxidizing agents. Herein, we used the NBT assay system to analyze the scavenging of superoxide radicals by crude and purified extracts of coriander. In the system, xanthine oxidase, which is one of the main enzymatic sources of ROS *in vivo*,

generated superoxide radicals, which consequently reduced NBT to yield insoluble blue formazan. As shown in Figure 6, a dose-dependent study was done towards scavenging superoxide radicals by the crude extracts 28.2±1.2% and the purified extracts as 59.2±1.4% inhibits NBT reduction at a maximum dosage of 50 µl. The scavenging efficacy of purified extract was dose dependent with IC_{50} value of 37.57 µg/ml. This observation indicates that the purified extract is more efficient superoxide scavenger in comparison with crude extract

Furthermore, the ferric ion chelating activity of crude and purified extracts of coriander along with standard metal ion chelator Ethylene diamine tetra acetic acid (EDTA) was tested as summarized in Figure 7. The ferrous ion chelating effect of extracts were more at 700 nm; OD indicating that it effectively chelates iron when compared to EDTA. Fenton reaction could cause damage to all the classes of biological molecules. Ferrous ions in excess may result in cellular damage. When iron undergoes Fenton reaction, reduced metals form highly reactive oxygen radicals and thereby contributing oxidative stress. The reducing power of crude and purified extract of tested are shown in Figure 8. As shown in the figures, the purified extract shows more promising reducing power in comparison to crude extract.

Oxidation of oils had great influence on the shelf life of ingredients and finished products. The stability of commercially available sunflower oil with or without the addition of crude and purified extracts isolated from coriander leaves was measured using rancimat. The induction time was 4.31 h for sunflower oil; 6.25 h for sunflower oil mixed with crude extract and 7.06 h for sunflower oil mixed with purified extract isolated from coriander leaves. A curve was obtained when induction period was plotted against conductivity (Fig. 9).

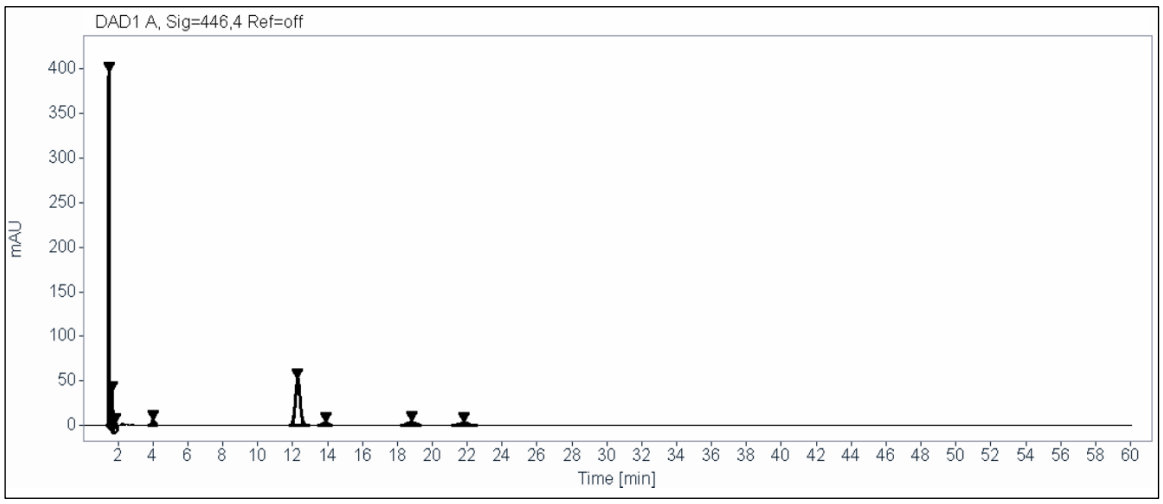


Fig. 1: *HPLC Profile of Crude Extract of Coriander Leaves.*

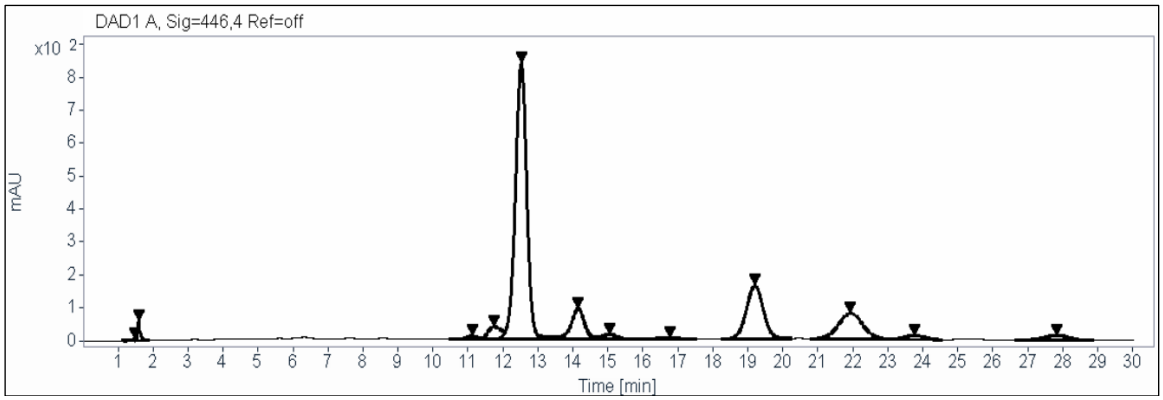


Fig. 2: *HPLC Profile of Oxycarotenoid Extract Isolated from Coriander Leaves.*

Table 1: *Summary of the Antioxidant Assays with IC_{50} Values.*

Sr. No	Antioxidant assays	Crude extract	Purified extract
1.	DPPH (1,1-diphenyl-2-picryl hydrazyl radical) assay:IC_{50} (µg/ml)	17.61	14.06
2	Hydroxyl Radical Scavenging Activity: :IC_{50} (µg/ml)	87.05	39.79
3.	Lipid peroxide Inhibition: :IC_{50} (µg/ml)	97.48	71.4l
4	Superoxide Activity:	113.7	37.57
5	Iron chelating activity:IC_{50} (µg/ml)	Nil	Nil

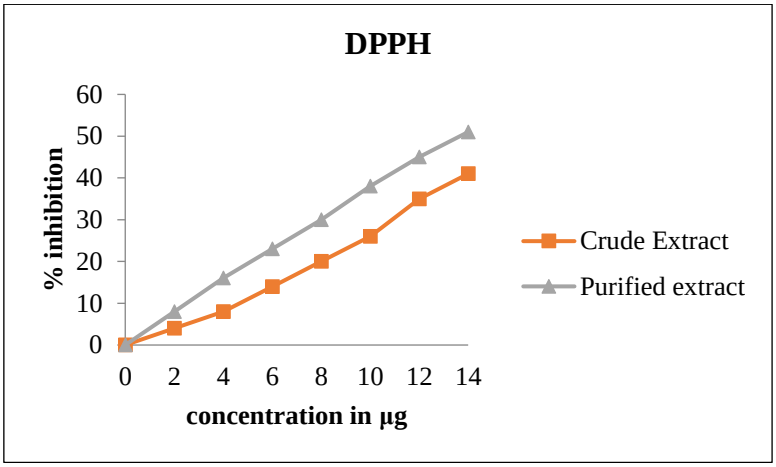


Fig. 3: *Free Radical Scavenging Activity of Coriander Extracts by DPPH Assay.*

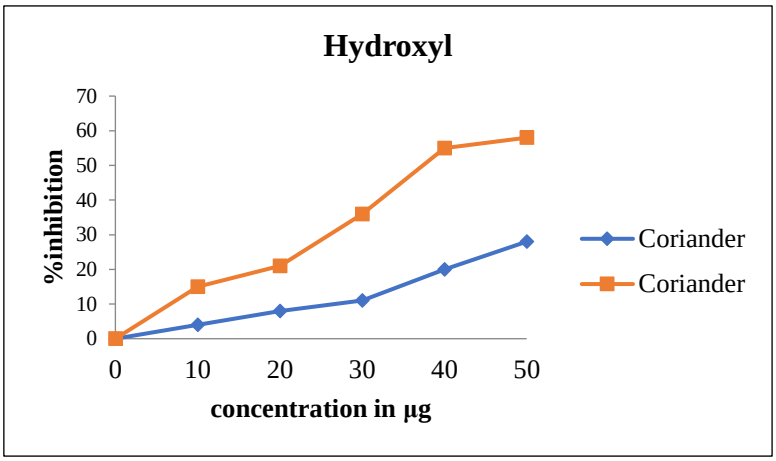


Fig. 4: *Hydroxyl Radical Scavenging Activity of Coriander Extracts.*

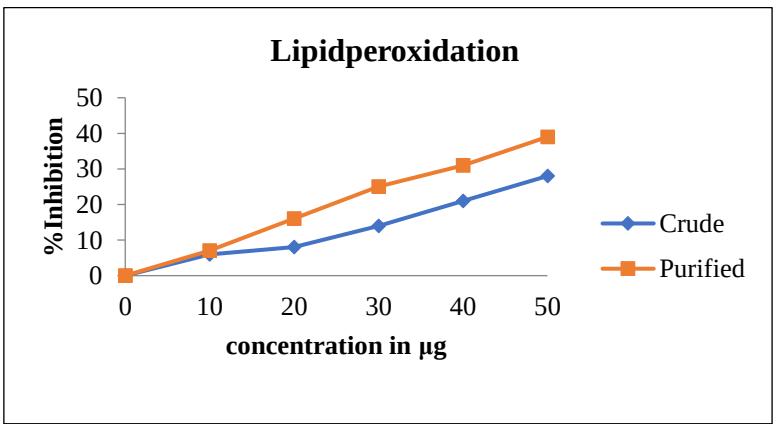


Fig. 5: *Lipid Peroxidation of Extracts Isolated from Coriander Leaves.*

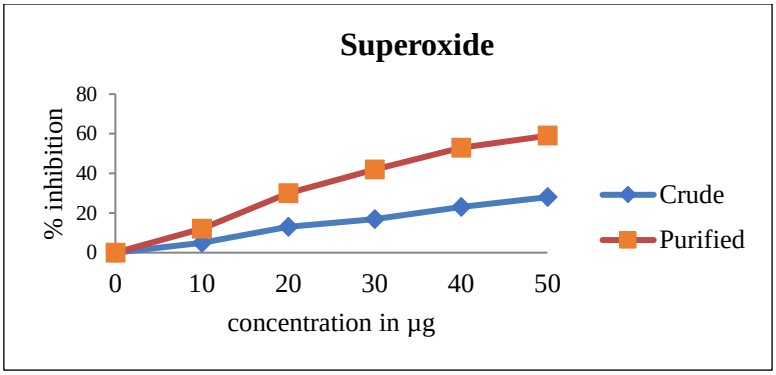


Fig. 6: *Dose-Dependent Superoxide Radical Scavenging Activity of Extracts Isolated from Coriander Leaves.*

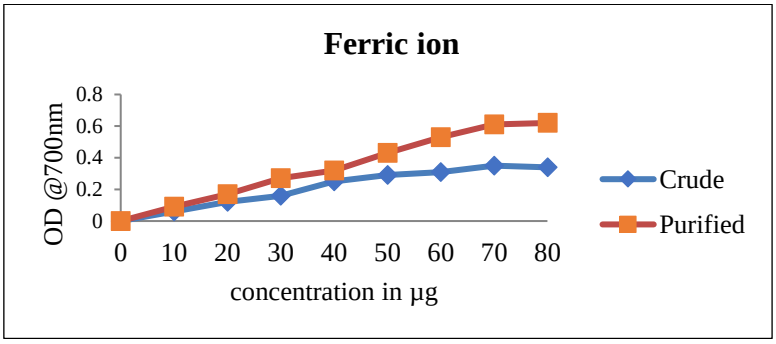


Fig. 7: *Ferric Ion Reducing Activity of Crude and Purified Extracts Isolated from Coriander Leaves.*

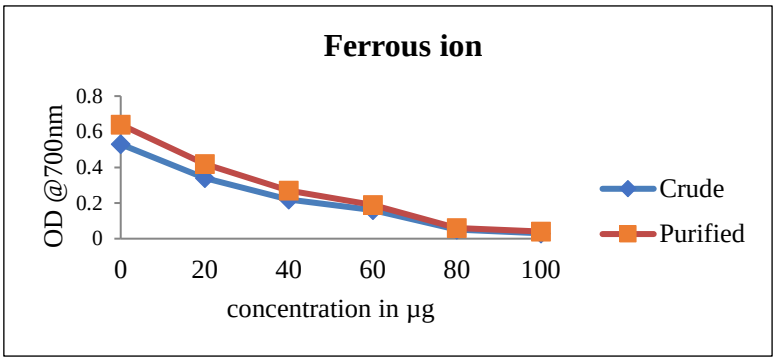
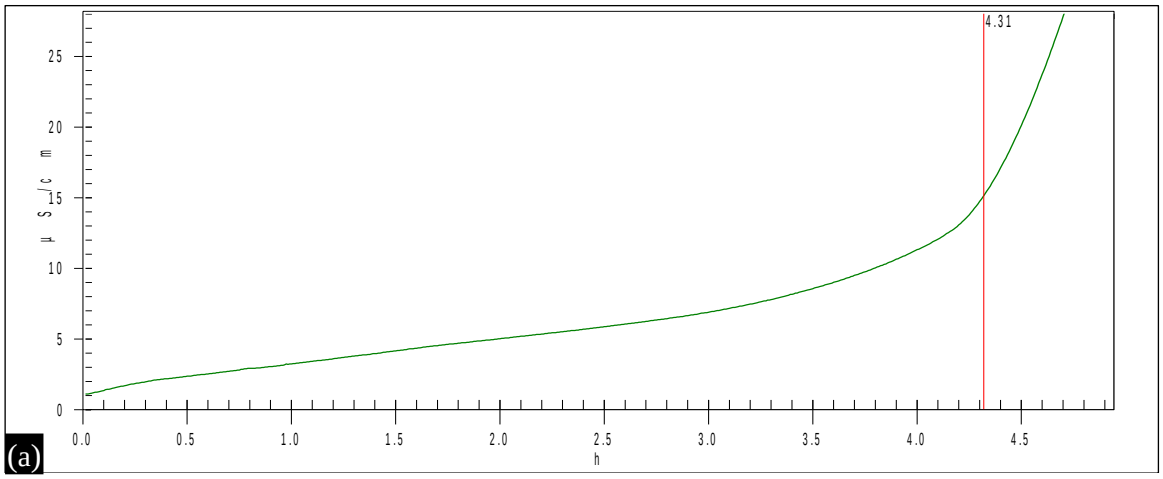


Fig. 8: *Ferrous Ion Chelating Activity of Crude and Purified Extracts Isolated from Coriander Leaves.*



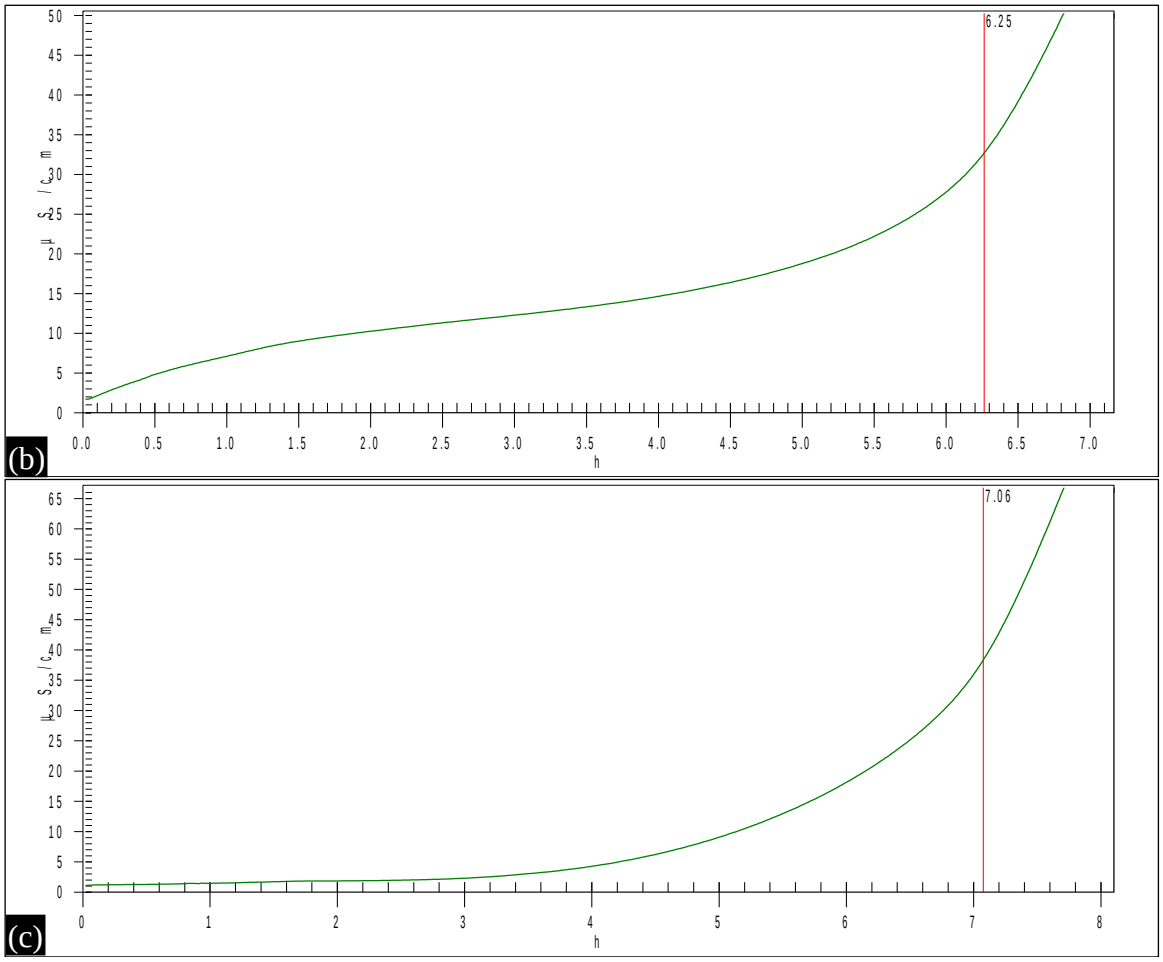


Fig. 9: *Oxidative Stability of Sunflower Oil by Rancimat Assay: a) Sunflower Oil as Such; b) Sunflower Oil with Crude Extract Isolated from Coriander Leaves; c) Sunflower Oil with Oxycarotenoid Extract Isolated from Coriander Leaves*.

CONCLUSION

The present study on biochemical studies of crude and purified oxycarotenoids extracts isolated from coriander leaves showed that the extracts prevents the free radical formation and the results proved that the oxycarotenoid extracts were more effective than crude extracts. Also the result on the oxidation stability shown by the rancimat assay has also proved that oxycarotenoid extracts are more effective than crude extracts. In conclusion coriander leaves as whole can be a good dietary antioxidant source.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Kancor Ingredients Ltd. for providing the facilities in the research division and Dr. Dinesha Ramadas, Scientific Officer at Adichunchanagiri Institute for Molecular Medicine for the guidance and support. Also we acknowledge Dr. T.K Sunilkumar, Technical Expert for his encouragement and support throughout.

REFERENCES

1. EI Gharras H. Polyphenois: Food sources, properties and applications—a review. *International Journal of Food Science & Technology*. 2009; 44: 2512–18p.
2. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharm Biomed Anal*. 2006; 41(5): 1523–42p.
3. Perumalla AVS, Hettiarachchy NS. Green tea and grape seed extracts—Potential applications in food safety and quality. *Food Research International*. 2011; 44(4): 827–39p.
4. Anand David AV, Arulmoli R, Parasuraman S. Overviews of Biological Importance of Quercetin: A Bioactive Flavanoid. *Pharmacognosy Reviews*. 2016; 10: 84–9p.

5. Khan D, McGrath KR, Dorosheva O, *et al*. Structural elements that govern Sec14-like PITP sensitivities to potent small molecule inhibitors. *J Lipid Res.* 2016; 57(4): 650–62p
6. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. *Food Analytical Methods.* 2009; 2(1): 41–60p.
7. Ugulu I, Baslar S, Yorek N, *et al*. The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey (Article). *Journal of Medicinal Plants Research.* 2009; 3(5): 345–67p.
8. Halliwell B, Gutteridge JMC (Eds.). *Free Radicals in Biology and Medicine*. Oxford: Clarendon Press; 1985. 107p.
9. Aquino R, Morelli S, Lauro MR, *et al*. Phenolic constituents and antioxidant activity of an extract of Anthurium versicolor leaves. *J Nat Products.* 2001; 64: 1019–23p.
10. Shimazaki UN, Mowri HO, Inoue K. Formation of age pigment like fluorescent substances during peroxidation of lipids in model membranes. *Biochem Biophysic Acta.* 1984; 792: 123–8p.
11. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the auto oxidants of polyunsaturated fatty acid methyl esters. *Arch Biochem Biophys.* 1962; 98: 253–61p.
12. Lee JC, Kim HR, Kim J, *et al*. Antioxidant activity of ethanol extract of the stem of *Opuntia ficus-indica* var. saboten. *J Agri Food Chem.* 2002; 50: 6490–6p.
13. Wang L, Yen JH, Ling HL, *et al*. Antioxidant Effect of Methanol Extracts from Lotus Plumule and Blossom (*Nelumbo nucifera* Gertn.). *Journal of Food and Drug Analysis*. 2003; 11(1): 60–6p.
14. Suter M, Richter C. Anti and Pro-oxidative properties of PADMA 28, a Tibetan herbal formulation. *Redox Report.* 2000; 5(1): 17–22p.
15. Laubli M, Bruttle P. Determination of the oxidative stability of fat and oils, comparison between an active oxygen method (Aocscd 12-57) and rancimat method. *J Am Oil Chem Soc.* 1986; 63: 792–5p.
16. Srinivas L, Shalini VK. DNA damage by smoke: protection by Turmeric and other inhibitors of ROS. *Free Radic Biol Med.* 1991; 11: 277–83p.

Cite this Article

Sherena PA, Annamala PT, Mukkadan JK, *et al*. Biochemical Assays of Crude and Purified Oxycarotenoid Extracts Isolated from Coriander Leaves and their Effect on Oxidative Stability of Oils by Rancimat Assay. *Research & Reviews: A Journal of Biotechnology*. 2018; 8(1): 1–10p.