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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF MICROENCAPSULATED AND NON-ENCAPSULATED ASTAXANTHIN

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ABSTRACT

The antioxidant properties of both encapsulated and non-encapsulated astaxanthin has been verified by various methods. Generally astaxanthin is considered as a powerful antioxidant compound when compared with other carotenoids. Encapsulation was carried out using sodium alginate, chitosan, Tripoly Phosphate (TPP) and liposomes. In our preliminary work, the encapsulated astaxanthin was synthesized by different methods and characterized. Further it mooted to study the antioxidant properties and radical scavenging. All the methods showed very good antioxidant activity for Microencapsulated method 4 (ME 4) (i.e liposomal encapsulated astaxanthin) when compared with non-encapsulated astaxanthin and other methods of encapsulation. Standard drug such as ascorbic acid, Gallic acid and BHT were used against the test samples to confirm the good antioxidant activity. Thus, it is concluded that both encapsulated and non-encapsulated astaxanthin showed better antioxidant activity than standard drug. Hence, it can be further explored for other pharmacological studies.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects. Astaxanthin is the main carotenoid pigment found in aquatic animals and is present in many of our favorite seafood's including salmon, trout, red seabream, shrimp, lobster, crab shell and fish eggs. It is also present in birds such as flamingoes and quails. Astaxanthin was first isolated in 1938 from American lobster. In many of the aquatic animals in which it is found, astaxanthin has several essential biological functions. The potent antioxidant and anti-inflammatory activity of astaxanthin were demonstrated in both experimental and human studies (R. T. Lorenz and G. R. Cysewski, 2000). Astaxanthin is chemically 3,30-dihydroxy-β, β -carotene-4, 40-dione a naturals pigments which has been widely used in feed as colorant approved by US FDA for specific use in animal and fish food (Vikram Sharma and Subash Chand, 2014). Astaxanthin's powerful antioxidant activity has been demonstrated in numerous studies showing the detrimental effects of free-radical-induced oxidative stress (Y. M. A. Naguib, 2000) and its potential to target many important health conditions. It has powerful antioxidant which is 10 times more capable than other carotenoids (Uma Nath Usha Kumari and Ravi Ramanujan, 2013). In terms of antioxidant power or potency, astaxanthin is 550 times stronger than vitamin E, and 6,000 times stronger than vitamin C. Recent studies have shown enhanced immune response and decreased DNA damage in human subjects following Astaxanthin administration (B. P. Chew and J. S. Park, 2003). Astaxanthin is capable of crossing the blood-brain barrier in mammals (M. O. M. Tso and T. T. Lam, 1996), a unique and important property in the realm of antioxidants. This characteristic allows Astaxanthin to extend its superior antioxidant activity to the central nervous system, which, being rich in unsaturated fatty acids is highly susceptible to oxidative damage by ROS (F. Facchinetti *et al,* 1998). The efficacy of astaxanthin in limiting the damage produced by ROS-induced oxidative stress and improving health parameters in the tissues and the body was demonstrated in a series of *in-vitro* experiments, in pre-clinical studies and in human models. To our knowledge,

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the antioxidant & radical scavenging activity of microencapsulated astaxanthin was not yet demonstrated. Thus, in this paper, we have compared the *in vitro* antioxidant activity of encapsulated and non-encapsulated astaxanthin.

MATERIALS AND METHODS

Microencapsulation of astaxanthin using different agents

Astaxanthin purchased from Rudra Bio ventures Pvt Ltd, Bangalore was encapsulated using four different agents by ionotropic gelation method. In first method (Microencapsulated method 1 (ME 1)), microencapsulated astaxanthin was encapsulated by using sodium alginate and calcium chloride (S. F. Lin *et al,* 2016; S. A. Park *et al,* 2014; V. Suganya and S. T. Asheeba, 2015). In second method (Microencapsulated method 2 (ME 2)), microencapsulated astaxanthin was prepared using sodium alginate and chitosan (W. Krasaekoopt *et al,* 2006). In third method (Microencapsulated method 3 (ME 3)), chitosan – Tripolyphosphate was used to produce microencapsulated astaxanthin (Phathanee Thamaket and Patcharin Raviyan, 2015; L. Yangchao *et al,* 2011). In fourth method (Microencapsulated method 4 (ME 4)), liposome encapsulated astaxanthin was carried out by the method followed by C. H. Chiu *et al,* 2016. The *in vitro* antioxidant activity such as total antioxidant activity, DPPH radicals scavenging activity, Hydrogen peroxide scavenging activity, Nitric oxide scavenging activity, Ferric reducing antioxidant power, Deoxyribose scavenging activity, ABTS cation radical scavenging activity, Superoxide radical scavenging activity (SO), LPO(egg yolk), SOD and β – carotene linolenic acid assay were performed for both microencapsulated and non – encapsulated astaxanthin. The detailed procedure was given below.

In vitro antioxidant activity of microencapsulated and non-encapsulated astaxanthin

Total antioxidant activity

The total antioxidant capacity of samples was evaluated by P. Prieto *et al,* 1999. Total antioxidant capacity (TAC) reagent were prepared as follows: 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2356 g of ammonium molybdate (4mM solution) was dissolved in distilled water and made up to 250 ml. 300 µl of test samples were dissolved in 3 ml of TAC reagent. Reaction mixture was incubated at 95° C for 90 minutes. All the samples were measured at 695 nm and ascorbic acid was used as standard (P. B. Kasangana *et al,* 2015; A. B. Aliyu *et al,* 2012; R. S. Phatak and A. S. Hendre, 2014). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples.

DPPH radical scavenging assay

The scavenging activity of non-encapsulated and encapsulated astaxanthin for DPPH radical were determined by the method of G. H. Yen and H. Y. Chen, 1995. Briefly, 2.0 ml of test samples were mixed with 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm (M. R. A. Manimala and R. Murugesan, 2014; A. Y. Loo *et al,* 2008). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples. The control was tested without standard and test samples. The scavenging effect (%) was calculated by using the formulae:

Scavenging effect (%) = (Absorbance of control –Absorbance of test solution)/Absorbance of control] × 100

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out by the procedure of R. J. Ruch *et al,* 1989. A solution of hydrogen peroxide (H_2O_2, 10 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). 3.4 ml of phosphate buffer was mixed with 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM) and 1ml (0.25 mg) of test solution was added to it. The absorbance value of the reaction mixture was recorded at 230 nm after 10 minutes incubation at room temperature. Blank solution contains sodium phosphate buffer without H_2O_2. Ascorbic acid was used as the standard (D. Gulcin, 2006; M. Elmastas *et al,* 2005). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples. Control solution containing buffer and H_2O_2 were taken. The percentage of H_2O_2 scavenging were calculated using the following equation:

H_2O_2 scavenging effect (%) = (Absorbance of control – Absorbance of test solution)/Absorbance of control) × 100

Nitric oxide scavenging activity

Nitric oxide scavenging activity was performed as follows: 3ml of 10 mM of sodium nitroprusside was prepared in phosphate buffer saline (pH 7.4, 0.2 M) and mixed with 1 ml of test solution and incubated at 25°C for 180 mins. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. The test solution was mixed with an equal volume of freshly prepared Griess reagent. The absorbance was measured at 546 nm. Ascorbic acid was used as the positive control. The percentage inhibition of the test and standard was calculated and recorded (Fadzai Boora *et al,* 2014). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples. Control was tested against test solution.

The percentage nitric oxide radical scavenging activity of both test sample and gallic acid were calculated using the following formula:

Nitric oxide activity (%) = (Absorbance of control-Absorbance of test) / Absorbance of control × 100.

Ferric reducing antioxidant Power (FRAP)

Reducing power of test samples was determined by the method prescribed by M. Oyaizu, 1986. Briefly, 1.0 mL of test sample was mixed with 2.5 ml of Phosphate buffer (0.2 M, pH 6.6) and 2.5 mL Potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of Trichloroacetic acid (10%) was added and centrifuged (650 g) for 10 min. From the upper layer, 2.5 mL solution was mixed

with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Increased absorbance indicate increased reducing power (Karunamoorthy Manivannan, Perumal Anantharaman and Thangavel Balasubramanian, 2012). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples.

Deoxyribose Radical Scavenging Activity

Deoxyribose non-site specific hydroxyl radical scavenging activity of test solution was estimated. Briefly, 2.0 ml aliquots of test samples were added to the test tube containing reaction mixture of 2.0 ml $FeSO_4.7H_2O$ (10mM), 0.2 ml EDTA (10mM) and 0.2 ml deoxyribose (10mM). The volume was made up to 1.8 ml with phosphate buffer (0.1M, pH-7.4) and to that 0.2 ml H_2O_2 (10mM) was added. The mixture was incubated at 37°C under dark for 4 hours. After incubation, 1 ml of TCA (2.8%) and TBA (1%) were added to the mixture, and kept in boiling water bath for 10 min. After treatment absorbance was measured at 532nm. If the mixture was turbid, the absorbance was measured after filtration. Ascorbic acid was used as standard (H. Indu and R. Seenivasan, 2013). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples. Control tube was also measured containing only reagents. Scavenging activity (%) was calculated using the equation:

Deoxyribose radical scavenging activity (%) = (Absorbance of control - Absorbance of test) / Absorbance of control × 100

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Assay

Free radical scavenging activity was also determined by ABTS radical cation decolorization assay (R. Re *et al,* 1999. ABTS radical cation was generated by mixing 20mM ABTS solution with 70mM potassium peroxodisulphate and allowing it to stand in dark at room temperature for 24 hours before use. 0.6 ml of test samples (0.25 mg) were mixed with 0.45 ml of ABTS reagent and absorbance of these solutions was measured at 734 nm after 10 min of incubation. The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples along with the control.
ABTS radical cation scavenging assay [%] = (Absorbance of control - Absorbance of test) / Absorbance of control × 100

Superoxide radical scavenging activity (SO)

Scavenging of superoxide radical was studied using the method elaborated by C. C. Winterbourn *et al,* 1975. Assay tubes contained 0.2 ml of the test samples (corresponding to 20 mg extract) with 0.2 ml EDTA (12mM), 0.1 ml Nitro blue tetrazolium, 0.05 ml riboflavin (20µg) and 2.64 ml phosphate buffer (50 mM, 7.6 pH) were taken. The control tube was set up with DMSO (Dimethyl sulfoxide) solution instead of the test solution. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 mins. A560 was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition by the test samples was calculated by comparing with O.D of the control tubes (M. Pandithurai and S. Murugesan, 2014). The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples.

% Inhibition= (Absorbance of control- Absorbance of test) / Absorbance of control × 100

Estimation of lipid peroxidation using egg yolk

Inhibitions of lipid peroxidation in the egg of hen were determined using a modified method thiobarbituric acid-reactive species (TBARS) assay (E. S. Adithya *et al,* 2013; G. Ruberto *et al,* 2000; J. A. Badmus *et al,* 2013). Egg homogenate (0.5 ml, 10% in distilled water, v/v) and 0.1 ml of test samples were mixed separately in a test tube and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture to induce lipid peroxidation and incubated for 30 min. Subsequently, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA (w/v) in 1.1% sodium dodecyl sulfate (SDS) and 0.05 ml 20% TCA was added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples along with the control tube.

% Inhibition= (Absorbance of control- Absorbance of test) / Absorbance of control × 100

Superoxide Dismutase Scavenging Assay (SOD)

Measurement of superoxide anion scavenging activity of test samples along with the standard was performed based on the method described by M. Nishimiki *et al,* 1972, with slight modifications. About 1ml of Nitroblue Tetrazolium (NBT) solution containing 156µM NBT dissolved in 1.0 ml of phosphate buffer (100mM, pH 7.4) and 1ml of NADH solution containing 468 µM of NADH which is dissolved in 1ml of phosphate buffer (100 mM, pH 7.4) with 0.1 ml of various concentrations of test samples (200 to 1000 µg/ ml) were mixed and the reaction was started by adding 100 µl of Phenazine methosulphate (PMS) solution containing 60 µM of PMS 100 µl of phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560nm was measured against the control samples. BHT was used as the reference compounds (200 to 1000 µg/ ml). The percentage inhibition was calculated by comparing the results of control and test samples.

% of SOD = (Absorbance of control- Absorbance of test) / Absorbance of control × 100

β carotene linoleic acid assay

β- Carotene linoleic acid assay was performed based on H. E. Miller, 1971; M. Zargar *et al,* 2011. Briefly, in 10 ml of chloroform, 2 mg β-carotene, 200 mg linoleic acid and 20 mg Tween 40 were dissolved which was taken in flask. Chloroform was evaporated using vacuum evaporator apparatus. Then, 50 ml of distilled water saturated with oxygen by shaking for 30 mins. This mixture is used as stock solution. 200 µl of test samples were mixed with 2.5 ml of stock solution in the test tube. Afterwards, the samples were placed in an oven at 50°C for 3 hours. The absorbance was read at

470 nm. The varied concentration in the range of 200 to 1000 µg/ ml were taken for both standard and test samples.
The percent of antioxidant activity was calculated from the following equation:

% of antioxidant activity = (Absorbance of control-Absorbance of test) / Absorbance of control × 100

Statistical analysis

The statistical analyses for all the experiments were done using Excel 2013 through statistical formula. Experimental data were expressed as mean ± SD and IC 50 values were calculated. The experiment was performed in triplicates for all the test samples.

RESULTS AND DISUCUSSION

In vitro Antioxidant activity of microencapsulated and non-encapsulated astaxanthin

Total antioxidant activity

The total antioxidant activity of standard ascorbic acid and test samples were measured in the concentration of 200 – 1000 µg/ml and the OD values were noted in the Table 1 and Table 2. Figure 1 and Figure 2 denotes, the total antioxidant activity of Ascorbic acid standard curve, encapsulated and non-encapsulated astaxanthin compound.

The total antioxidant activity of non-encapsulated astaxanthin (0.007 ± 0.189 to 0.033 ± 0.002) and ME 4 (0.006 ± 0.002 to 0.031 ± 0.159) indicates higher activity whereas, ME 2 (0.003 ± 0.198 to 0.015 ± 0.215) and ME 3 (0.001 ± 0.001 to 0.009 ± 0.189) indicates lower activity and ME 1 (0.005 ± 0.115 to 0.025 ± 0.003) indicates moderate activity when compared with standard ascorbic acid (0.005 ± 0.008 to 0.024 ± 0.538).

DPPH radical scavenging assay

In the present study, the DPPH activity of ascorbic acid, non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 were determined and the results (OD values and percentage) are presented in Table 3 and Table 4. DPPH radical scavenging activities (%) of standard ascorbic acid and different test samples with different concentration (200 – 1000 µg/ml) are represented in Figure 3 and Figure 4. All these samples possessed the ability to scavenging DPPH at various degrees, with the ME 4 (28.73% to 91.78%) was found to be the most potent scavenger followed by ME 2 (25.90% to 90.45%) and ME 3 (26.28% to 90.55%). The other test samples non-encapsulated astaxanthin and ME 1 showed the minimum DPPH radical scavenging activity i.e. 25.61% to 89.79% and 25.33% to 89.41%. The scavenging effect of standard ascorbic acid was founded to be from 23.25% to 88.44% with IC 50 values of 594.036 µg/ml. The IC 50 values of test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3, ME 4 and ME 5 was 581.329, 588.835, 575.480, 573.108 and 546.558 µg/ml.

Table 1. Total antioxidant content of Ascorbic acid standard

Content	Concentration (µg/ml)	O.D at 695 nm Mean ± S.D
S1	200	0.005 ± 0.008
S2	400	0.011 ± 0.180
S3	600	0.016 ± 0.198
S4	800	0.020 ± 0.001
S5	1000	0.024 ± 0.538

Table 2. Total antioxidant content O.D values at 695 nm for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD	ME 1 Mean ± SD	ME 2 Mean ± SD	ME 3 Mean ± SD	ME 4 Mean ± SD
200	0.007 ± 0.189	0.005 ± 0.115	0.003 ± 0.198	0.001 ± 0.001	0.006 ± 0.002
400	0.014 ± 0.197	0.010 ± 0.158	0.007 ± 0.002	0.003 ± 0.003	0.012 ± 0.005
600	0.020 ± 0.205	0.015 ± 0.189	0.009 ± 0.001	0.005 ± 0.115	0.018 ± 0.001
800	0.027 ± 0.291	0.020 ± 0.015	0.012 ± 0.018	0.007 ± 0.158	0.024 ± 0.108
1000	0.033 ± 0.002	0.025 ± 0.003	0.015 ± 0.215	0.009 ± 0.189	0.031 ± 0.159

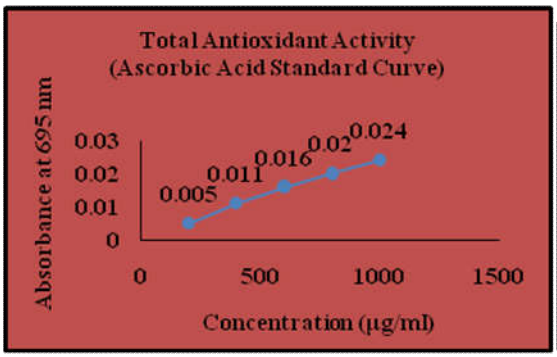

Figure 1. Total antioxidant activity of Ascorbic acid standard curve

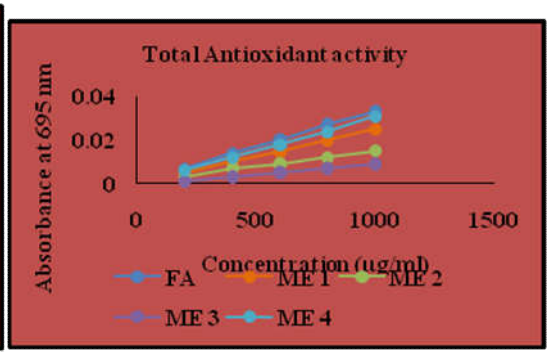

Figure 2. Total antioxidant activity of encapsulated and non-encapsulated astaxanthin

Table 3. DPPH radical scavenging assay of Ascorbic acid standard

Content	Concentration (µg/ml)	Percentage Mean ± S.D	IC 50 Values
S1	200	23.25 ± 0.104	
S2	400	33.65 ± 0.259	
S3	600	46.79 ± 0.121	
S4	800	60.21 ± 0.035	594.036
S5	1000	88.44 ± 0.083	

Table 4. DPPH radical scavenging assay Percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	25.61 ± 0.035	25.33 ± 0.133	25.90 ± 0.061	26.28 ± 0.071	28.73 ± 0.101
400	34.69 ± 0.031	34.03 ± 0.080	34.88 ± 0.015	35.35 ± 0.081	37.15 ± 0.076
600	44.90 ± 0.057	43.86 ± 0.108	45.37 ± 0.046	45.94 ± 0.319	47.64 ± 0.142
800	62.29 ± 0.053	61.72 ± 0.219	63.04 ± 0.150	63.23 ± 0.279	65.31 ± 0.083
1000	89.79 ± 0.057	89.41 ± 0.137	90.45 ± 0.095	90.55 ±0.189	91.78 ± 0.074
IC 50 Values	581.329	588.835	575.480	573.108	546.558

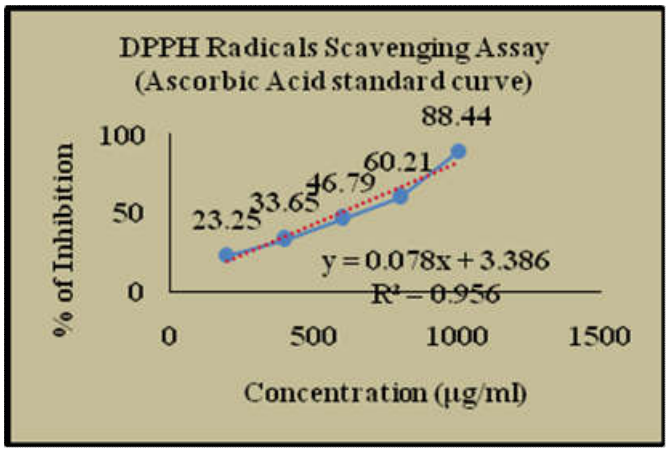

Figure 3 DPPH radical scavenging assay of Ascorbic acid standard

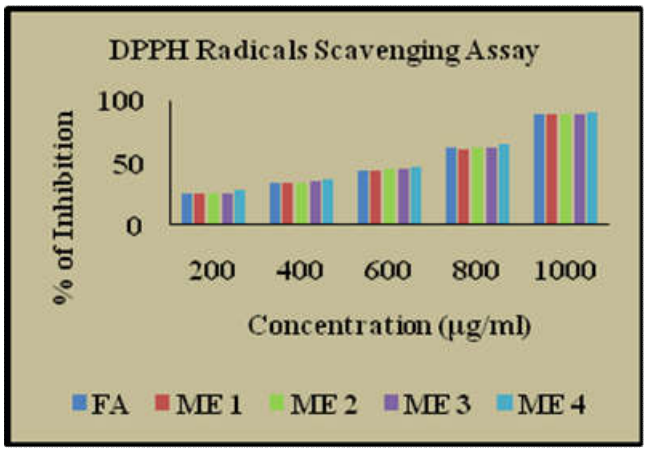

Figure 4 DPPH radical scavenging assay of encapsulated and non-encapsulated astaxanthin

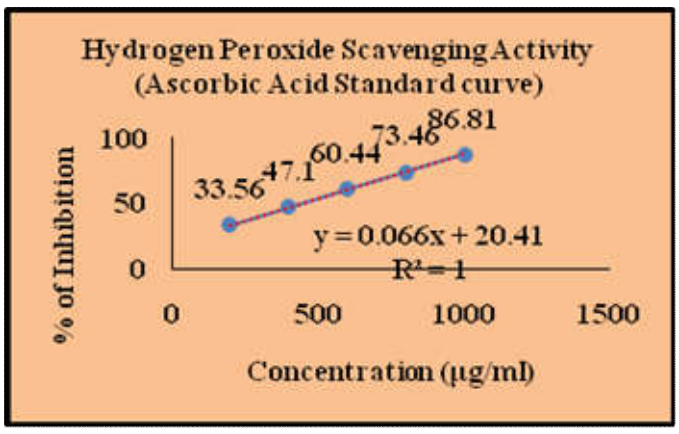

Figure 5. Hydrogen peroxide scavenging activity of Ascorbic acid standard curve

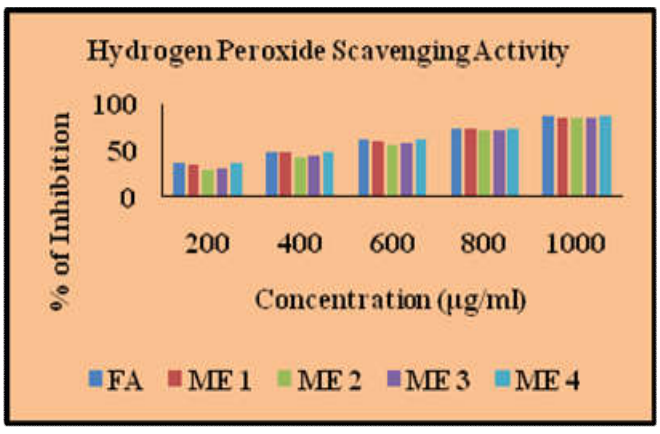

Figure 6. Hydrogen peroxide scavenging activity of encapsulated and non-encapsulated astaxanthin

Hydrogen peroxide scavenging assay

The hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to the cells because rise in hydroxyl radicals in the cells. The H_2O_2 radical scavenging was performed and the values were recorded. The percentage and IC 50 values of each samples were also calculated and entered in the Table 5 and Table 6. Based on the percentage and concentration in the range of 200 – 1000 µg/ml the Figure was plotted (Figure 5 and Figure 6).

The maximum scavenging of test samples for concentration 200 - 1000µg/ml was shown by ME 4 (37.42% to 87.60%) and non-encapsulated astaxanthin (37.09% to 87.44%) followed by ME 1 (35.13% to 87.18%) and ME 3(31.22% to 86.29%). The minimum activity was founded in ME 2 with 29.57% to 85.68% inhibition. Similarly, the standard ascorbic acid possesses 33.56% to 86.81% of inhibition.

The IC 50 values of both standard and test samples (Non-encapsulated astaxanthin, ME1, ME2, ME3 and ME4) were calculated and noted in Table 5 and Table 6 based on their percentage of inhibition.

Nitric oxide scavenging assay

The nitric oxide scavenging assay was performed with encapsulated and non-encapsulated astaxanthin samples along with the standard. The Percentage of inhibition and IC 50 values were noticed in Table 7 and Table 8. Based on percentage of inhibition and different concentration ranges 200 – 1000 µg/ml the Figure 7 and Figure 8 was plotted.

Ferric reducing antioxidant power

Ferric reducing antioxidant power was performed for the test samples such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 along with the standard ascorbic acid. The values obtained were recorded in Table 9 and Table 10. Each samples were tested with different concentration in the range of 200 – 1000 µg/ml. From the obtained OD values the Figure was drawn against different concentration which was presented in Figure 9 and Figure 10. Each test samples are related with standard which shows similar increasing in OD values for different concentration. Standard in the range of 0.010 to 0.050 was recorded.

Table 5. Hydrogen peroxide scavenging assay of Ascorbic acid standard

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	33.56 ± 0.217	
S2	400	47.10 ± 0.072	
S3	600	60.44 ± 0.079	
S4	800	73.46 ± 0.061	445.341
S5	1000	86.81 ± 0.072	

Table 6. Hydrogen peroxide scavenging assay Percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	37.09 ± 0.053	35.13 ± 0.091	29.57 ± 0.047	31.22 ± 0.240	37.42 ± 0.110
400	49.63 ± 0.050	48.29 ± 0.212	43.46 ± 0.059	45.19 ± 0.115	49.82 ± 0.105
600	62.17 ± 0.075	61.17 ± 0.132	57.53 ± 0.196	58.53 ± 0.261	62.44 ± 0.083
800	74.88 ± 0.083	73.74 ± 0.326	71.65 ± 0.316	72.65 ± 0.091	75.16 ± 0.114
1000	87.44 ± 0.076	87.18 ± 0.125	85.68 ± 0.266	86.29 ± 0.251	87.60 ± 0.334
IC 50 Values	405.605	428.607	492.059	471.487	401.305

Table 7. Nitric oxide scavenging assay of Ascorbic acid standard

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	29.01 ± 0.080	
S2	400	43.34 ± 0.129	
S3	600	57.51 ± 0.188	494.868
S4	800	71.82 ± 0.170	
S5	1000	85.50 ± 0.263	

Table 8. Nitric oxide scavenging activity Percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	41.16 ± 0.176	38.69 ± 0.211	35.57 ± 0.163	39.52 ± 0.187	44.21 ± 0.188
400	53.24 ± 0.070	51.23 ± 0.155	48.76 ± 0.150	51.72 ± 0.311	55.27 ± 0.100
600	65.20 ± 0.163	63.58 ± 0.117	61.30 ± 0.189	63.42 ± 0.269	66.46 ± 0.098
800	76.51 ± 0.194	75.75 ± 0.110	74.56 ± 0.112	75.78 ± 0.091	77.67 ± 0.158
1000	88.28 ± 0.105	87.68 ± 0.129	87.44 ± 0.380	87.84 ± 0.185	88.54 ± 0.234
IC 50 Values	346.779	381.453	422.047	373.72	304.124

The nitric oxide scavenging activity was done for test samples along with standard ascorbic acid. The highest inhibition of 44.21% to 88.54% was shown by the test sample ME 4 followed by non-encapsulated astaxanthin with inhibition of 41.16% to 88.28%. The moderate inhibition was founded in ME 3 and ME 1 with percentage of 39.52% to 87.84% and 38.69% to 87.68%. The lowest inhibition was recorded in ME 2 (35.57% to 87.44%). All the test samples possesses higher percentage of inhibition when compared with standard ascorbic acid which produced only 29.01% to 85.50%.

Non-encapsulated astaxanthin showed 0.011 to 0.055, ME 1 showed 0.013 to 0.065, ME 2 showed 0.012 to 0.063, ME 3 showed 0.010 to 0.052 and ME 4 showed 0.015 to 0.74. Test samples exhibited more reducing activity than the standard one.

Deoxyribose radical scavenging assay

In this assay, the antioxidant activity was determined based on the ability of the antioxidant components in the samples to inhibit deoxyribose oxidation by reactive OH^- generated from Fenton's type reaction.

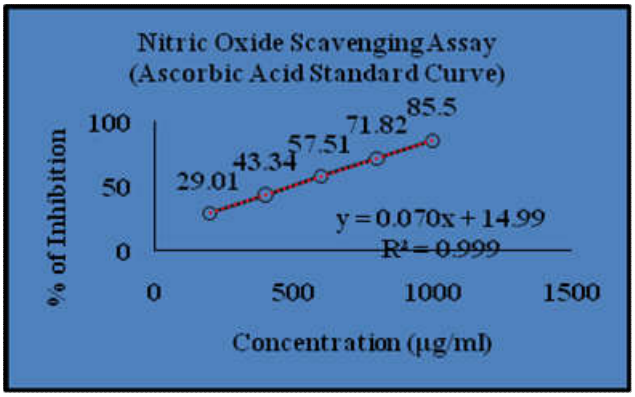

Figure 7. Nitric oxide scavenging assay of ascorbic acid standard

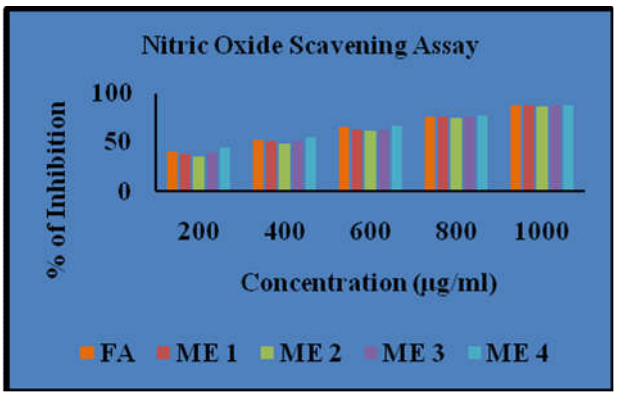

Figure 8. Nitric oxide scavenging assay of encapsulated and non–encapsulated astaxanthin

Table 9. Ferric reducing antioxidant power of Ascorbic acid standard

Content	Concentration (µg/ml)	O.D at 700 nm Mean ± SD
S1	200	0.010 ± 0.198
S2	400	0.020 ± 0.211
S3	600	0.031 ± 0.179
S4	800	0.041 ± 0.201
S5	1000	0.050 ± 0.003

Table 10. Ferric reducing antioxidant O.D values at 700 nm for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Mean ± SD	ME 1 Mean ± SD	ME 2 Mean ± SD	ME 3 Mean ± SD	ME 4 Mean ± SD
200	0.011 ± 0.151	0.013 ± 0.118	0.012 ± 0.003	0.010 ± 0.001	0.015 ± 0.018
400	0.021 ± 0.139	0.027 ± 0.189	0.024 ± 0.118	0.021 ± 0.019	0.030 ± 0.131
600	0.033 ± 0.132	0.039 ± 0.231	0.035 ± 0.131	0.032 ± 0.119	0.045 ± 0.231
800	0.044 ± 0.001	0.052 ± 0.212	0.046 ± 0.153	0.041 ± 0.231	0.061 ± 0.283
1000	0.055 ± 0.151	0.065 ± 0.003	0.063 ± 0.159	0.052 ± 0.153	0.074 ± 0.003

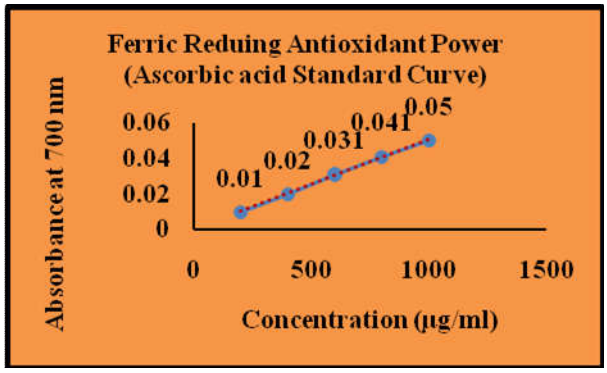

Figure 9. Ferric reducing antioxidant power Ascorbic acid standard curve

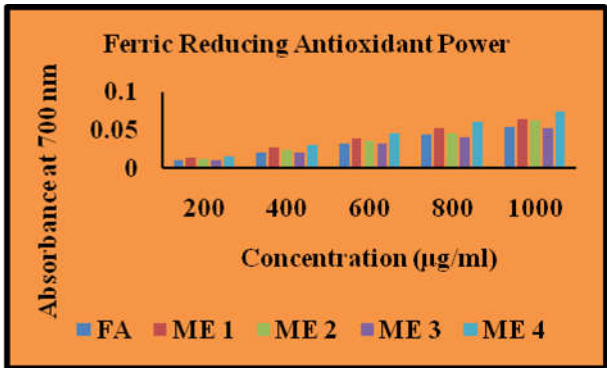

Figure 10. Ferric reducing antioxidant power of encapsulated and non-encapsulated astaxanthin

Table 11. Deoxyribose scavenging assay of Ascorbic acid standard

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 values
S1	200	27.58 ± 0.320	
S2	400	51.40 ± 0.225	
S3	600	61.27 ± 0.185	449.590
S4	800	75.75 ± 0.152	
S5	1000	90.40 ± 0.243	

In the present study deoxyribose radical scavenging assay was determined by studying the competition between deoxyribose and the astaxanthin samples. Table 11 and Table 12 indicates, the Deoxyribose radical scavenging assay in which the values were noted.

From each OD, the percentage of different samples at different concentration was obtained based on control values. From the Figure 11 and Figure 12, it is concluded that the standard (27.58% to 90.40%) showed less activity when matched with the test samples.

Table 12. Deoxyribose scavenging assay percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	29.25 ± 0.194	28.14 ± 0.111	28.64 ± 0.050	29.71 ± 0.110	30.08 ± 0.211
400	47.76 ± 0.067	48.45 ± 0.067	48.96 ± 0.074	49.70 ± 0.067	49.98 ± 0.226
600	63.30 ± 0.316	62.42 ± 0.107	62.93 ± 0.051	63.81 ± 0.171	64.00 ± 0.595
800	82.32 ± 0.314	77.14 ± 0.146	79.00 ± 0.131	80.19 ± 0.116	80.56 ± 0.131
1000	90.88 ± 0.060	89.45 ± 0.175	89.87 ± 0.281	91.25 ± 0.101	91.53 ± 0.071
IC 50 Values	439.032	453.017	444.197	431.582	427.600

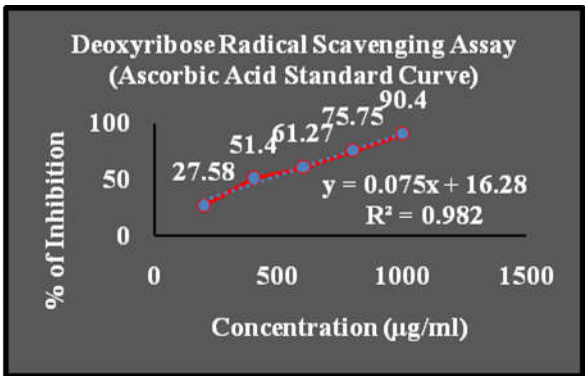

Figure 11 Deoxyribose Radical Scavenging Assay Ascorbic acid standard curve

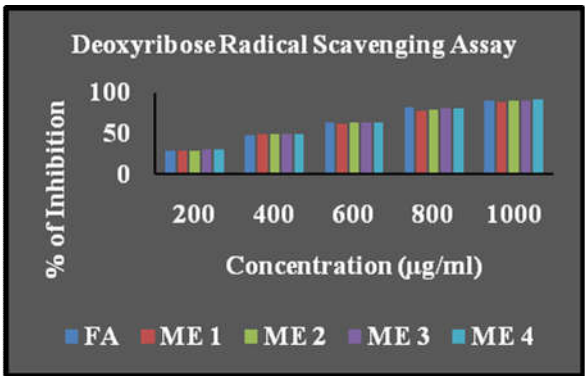

Figure 12 Deoxyribose Radical scavenging Assay of encapsulated and non-encapsulated astaxanthin

Table 13. ABTS radical cation scavenging assay of Gallic acid standard

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	26.06 ± 0.065	
S2	400	43.82 ± 0.081	
S3	600	62.79 ± 0.105	473.109
S4	800	77.74 ± 0.420	
S5	1000	92.51 ± 0.348	

Table 14. ABTS radical cation scavenging assay percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	25.43 ± 0.150	25.69 ± 0.061	25.62 ± 0.075	26.32 ± 0.060	26.84 ± 0.087
400	43.27 ± 0.239	43.45 ± 0.185	43.37 ± 0.117	44.04 ± 0.156	44.63 ± 0.311
600	62.31 ± 0.374	62.42 ± 0.042	62.35 ± 0.136	63.05 ± 0.067	63.68 ± 0.258
800	77.26 ± 0.046	77.48 ± 0.228	77.41 ± 0.074	78.04 ± 0.100	78.59 ± 0.192
1000	91.95 ± 0.089	92.30 ± 0.122	92.14 ± 0.131	92.69 ± 0.172	93.24 ± 0.185
IC 50 Values	479.734	477.214	478.166	470.121	463.325

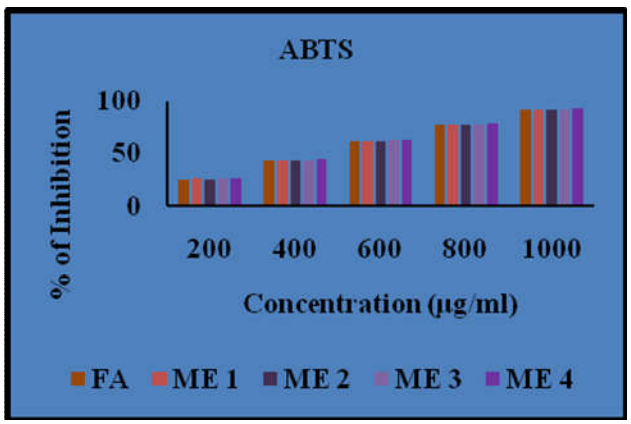

Figure 13 ABTS radical cation scavenging assay Gallic acid standard curve

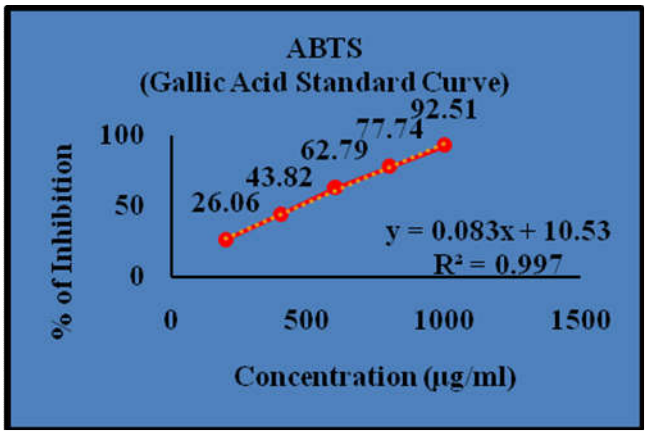

Figure 14 ABTS radical cation scavenging assay of encapsulated and non-encapsulated astaxanthin

Topmost percentage was gained for ME 4 (30.08% to 91.53%) and ME 3 (29.71% to 91.25%) with IC 50 values of 427.600 µg/ml and 431.582 µg/ml followed by non-encapsulated astaxanthin (29.25% to 90.82%) with IC 50 values of 439.032 µg/ml.

ME 1 (477.214 µg/ml) and ME 2 (478.166 µg/ml). The results of the present study indicate that the test samples exhibited higher ABTS radical activity than the standard.

Table 15. Superoxide radical scavenging activity of BHT standard curve

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	28.95 ± 0.222	
S2	400	46.80 ± 0.115	
S3	600	64.36 ± 0.153	440.260
S4	800	82.21 ± 0.219	
S5	1000	92.89 ± 0.344	

Table 16. Superoxide radical scavenging activity percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	36.70 ± 0.156	36.29 ± 0.111	35.70 ± 0.246	36.05 ± 0.110	37.23 ± 0.117
400	52.55 ± 0.156	52.20 ± 0.263	51.79 ± 0191	51.97 ± 0.086	52.97 ± 0.115
600	68.41 ± 0.115	68.12 ± 0.093	67.76 ± 0.106	67.94 ± 0.115	68.82 ± 0.091
800	84.09 ± 0.304	83.68 ± 0.100	83.32 ± 0.131	83.50 ± 0.104	84.44 ± 0.187
1000	93.58 ± 0.098	93.54 ± 0.115	93.24 ± 0.220	93.31 ± 0.122	94.01 ± 0.316
IC 50 Values	365.093	370.297	376.796	373.311	358.753

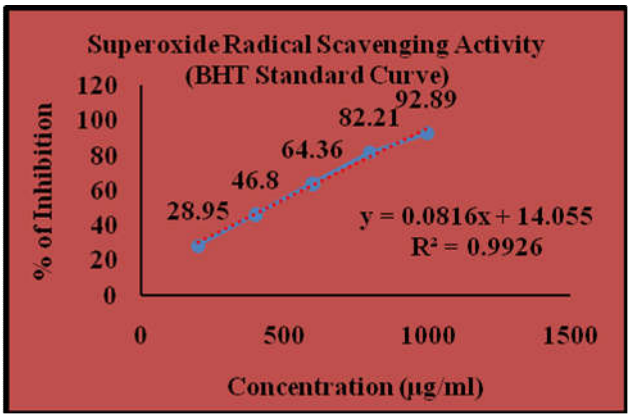

Figure 15. Superoxide radical scavenging activity BHT standard curve

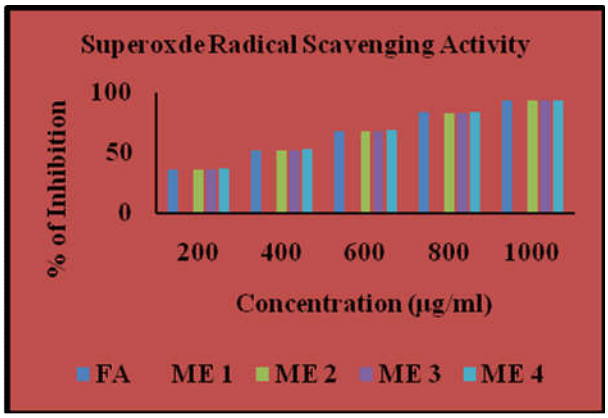

Figure 16. Superoxide radical scavenging activity of encapsulated and non-encapsulated astaxanthin

The lowest assay was seen in ME 1 (28.14% to 89.45%) with IC 50 of 453.017 µg/ml and ME 2 (28.64% to 89.87%) with IC 50 of 444.197 µg/ml.

ABTS radical cation-scavenging assay

In present study, the ABTS radical cation-scavenging assay performed showed that the antioxidant activity increases with increase in the concentration the values were noted in Table 13 and Table 14. Percentage and IC 50 values are calculated from OD values obtained for standard and test samples based on control values. Figure 13 and Figure 14 indicates the percentage of inhibition against concentration in the range of 200 – 1000 µg/ml for both gallic acid standard and test samples. The test samples such as non-encapsulated astaxanthin (91.95%), ME 1 (92.30%), ME2 (92.14%), ME 3 (92.69%) and ME 4 (93.24%) showed maximum % of inhibition at 1000 µg/ml concentration and they are slightly lower than that of the standard gallic acid (92.51%). The IC50 values of ABTS+ radical scavenging activity of standard was 473.109 µg/ml and its IC50 values were higher than that of ME 3 (470.121 µg/ml) and ME 4 (463.325 µg/ml) but lower than that of non-encapsulated astaxanthin (479.734 µg/ml),

Superoxide radical scavenging activity (SO)

Superoxide radical scavenging activity of test samples and the standard drug BHT was assessed which is recorded along with IC 50 values in Table 15 and Table 16. Decreasing in OD values tends to increase the percentage of inhibition. Figure 15 explain the superoxide radical scavenging activity of BHT which shows 28.95% at 200 µg/ml and 92.89% at 1000 µg/ml with IC 50 values of 440.26 µg/ml. All the test samples shows higher percentage of inhibition when compared with the standard BHT. The sample such as non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 exhibit 36.70%, 36.29 %, 35.70% 36.05% and 37.23% at concentration 200 µg/ml and 93.58%, 93.54%, 93.24%, 93.31% and 94.01% at 1000 µg/ml (Figure 16). But the IC 50 values of test samples (365.093 µg/ml, 370.297 µg/ml, 376.796 µg/ml, 373.311 µg/ml and 358.753 µg/ml) were founded to be lesser than the IC 50 Values of BHT (440.26 µg/ml).

LPO (Egg yolk)

Lipid peroxidase activity for non-encapsulated astaxanthin, ME 1, ME 2, ME 3 and ME 4 along with standard BHT was

performed and values are tabulated in Table 17 and Table 18. The IC values and percentage were also identified from the obtained OD values. Percentage of inhibition by standard BHT was founded to be 93.74% which is slightly lesser than the test samples non-encapsulated astaxanthin (94.28%), ME 1 (93.77%), ME 4 (94.47%) and slightly higher than that of other samples such as ME 2 (93.46%) and ME 3 (93.42%). The lowest IC 50 values were obtained for ME 4 (416.180 µg/ml) and non-encapsulated astaxanthin (418.944 µg/ml). Highest IC 50 values were obtained for ME 1 (469.586 µg/ml), ME 2 (497.257 µg/ml) and ME 3 (486.26 µg/ml) when compared with the standard BHT (473.23 µg/ml) (Figure 17 and Figure 18).

β- carotene linoleic acid assay: β carotene linoleic acid assay was carried out for both encapsulated and non-encapsulated astaxanthin along standard drug and data was entered in Table 21 and Table 22. Percentage of inhibition for standard BHT showed 19.60% at 200 µg/ml concentration and 91.17% at 1000 µg/ml. The test samples such as non-encapsulated astaxanthin (18.62%), ME 1 (19.51%), ME 2 (23.08%), ME 3 – 86.13% and ME 4 – 87.51%). The IC 50 values of Standard BHT (524.000 µg/ml) were similar to non-encapsulated astaxanthin (524.565 µg/ml) and ME 3 (525.037 µg/ml) and higher when compared with ME 1 (514.042 µg/ml), ME 2 (502.435 µg/ml) and ME 4 (515.535 µg/ml).

Table 17. Lipid peroxidase (egg yolk) activity of BHT standard curve

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	24.88 ± 0.228	
S2	400	43.41 ± 0.111	
S3	600	62.38 ± 0.119	473.230
S4	800	81.22 ± 0.105	
S5	1000	93.74 ± 0.096	

Table 18. Lipid peroxidase activity percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	31.33 ± 0.165	25.26 ± 0.873	21.44 ± 0.111	23.05 ± 0.119	31.55 ± 0.025
400	48.47 ± 0.140	43.88 ± 0.122	41.07 ± 0.253	42.24 ± 0.080	48.69 ± 0.075
600	65.63 ± 0.352	62.63 ± 0.047	60.73 ± 0.115	61.56 ± 0.137	65.86 ± 0.066
800	82.83 ± 0.125	81.35 ± 0.051	80.40 ± 0.091	80.68 ± 0.301	83.05 ± 0.087
1000	94.28 ± 0.100	93.77 ± 0.111	93.46 ± 0.151	93.42 ± 0.178	94.47 ± 0.055
IC 50 Values	418.944	469.586	497.257	486.260	416.180

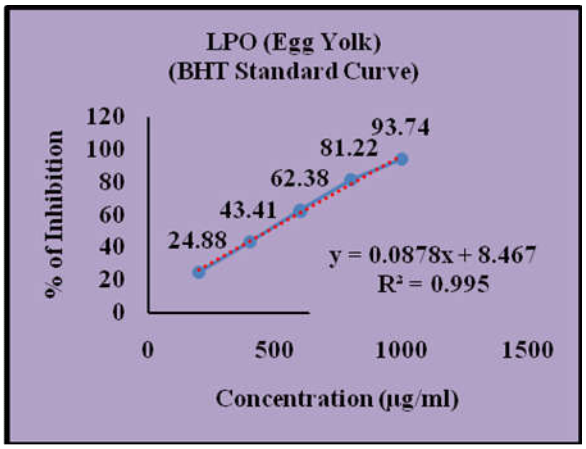

Figure 17. Lipid peroxidase activity BHT standard curve

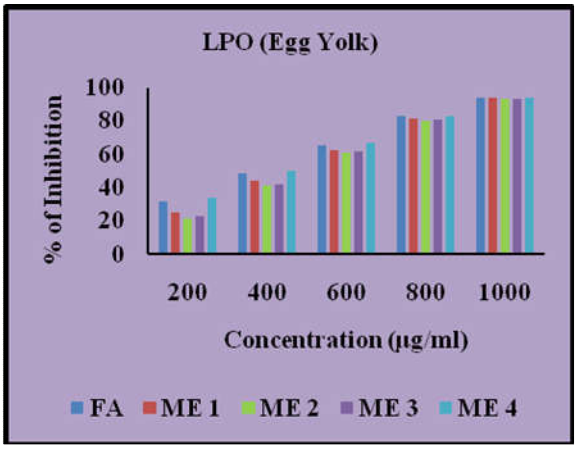

Figure 18. Lipid peroxidase activity of encapsulated and non-encapsulated astaxanthin

Table 19 Superoxide scavenging assay of BHT standard curve

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	17.75 ± 0.246	
S2	400	38.24 ± 0.115	
S3	600	58.78 ± 0.060	524.000
S4	800	79.41 ± 0.101	
S5	1000	91.77 ± 0.245	

Superoxide Dismutase scavenging assay (SOD)

When compared with standard, all the test samples showed low percentage of inhibition at 1000 µg/ml (Non-encapsulated astaxanthin - 85.16%, ME 1 - 85.44%, ME 2 – 85.26%, ME 3 (24.05%) and ME 4(16.19%) possess low value at 200 µg/ml and high values at 1000 µg/ml i.e. 92.63%, 93.34%, 93.60%, 93.68% and 94.01% when related with standard BHT (Figure 21 and Figure 22).

Table 20. Superoxide scavenging assay percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	26.22 ± 0.195	27.16 ± 0.140	28.55 ± 0.125	25.94 ± 0.247	26.60 ± 0.060
400	40.64 ± 0.101	41.66 ± 0.130	43.33 ± 0.079	40.46 ± 0.070	41.17 ± 0.110
600	55.48 ± 0.085	56.47 ± 0.229	57.37 ± 0.097	55.42 ± 0.132	55.94 ± 0.115
800	70.33 ± 0.078	70.52 ± 0.336	69.55 ± 0.161	70.18 ± 0.080	70.75 ± 0.130
1000	85.16 ± 0.095	85.44 ± 0.067	85.26 ± 0.076	86.13 ± 0.095	87.51 ± 0.151
IC 50 Values	524.565	514.042	502.435	525.037	515.535

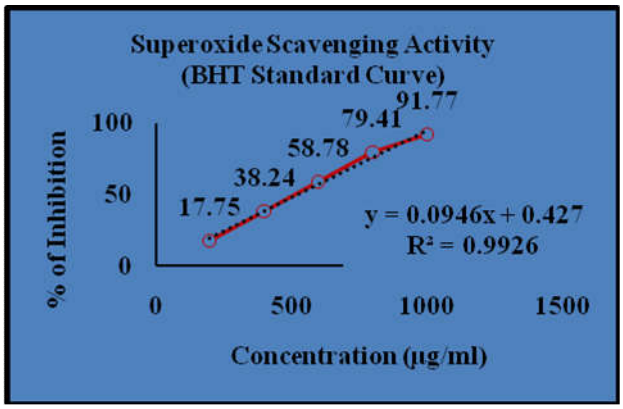

Figure 19. Superoxide scavenging activity BHT standard curve

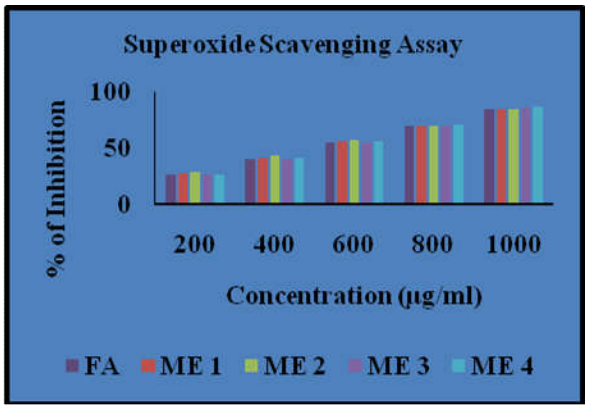

Figure 20. Superoxide scavenging assay of encapsulated and non-encapsulated astaxanthin

Table 21 β carotene linoleic acid assay of BHT standard

Content	Concentration (µg/ml)	Percentage Mean ± SD	IC 50 Values
S1	200	19.60 ± 0.159	
S2	400	52.23 ± 0.066	
S3	600	67.53 ± 0.155	454.934
S4	800	82.28 ± 0.101	
S5	1000	91.17 ± 0.093	

Table 22 β carotene linoleic acid percentage for different concentration of test samples

Concentration (µg/ml)	Non-encapsulated astaxanthin Percentage Mean ± SD	ME 1 Percentage Mean ± SD	ME 2 Percentage Mean ± SD	ME 3 Percentage Mean ± SD	ME 4 Percentage Mean ± SD
200	18.62 ± 0.085	19.51 ± 0.011	23.08 ± 0.150	24.05 ± 0.086	16.19 ± 0.122
400	41.30 ± 0.267	46.00 ± 0.125	48.58 ± 0.106	49.47 ± 0.080	49.88 ± 0.103
600	70.53 ± 0.131	73.12 ± 0.089	74.49 ± 0.183	74.90 ± 0.100	75.55 ± 0.227
800	85.26 ± 0.100	86.56 ± 0.142	87.21 ± 0.110	87.37 ± 0.090	88.02 ± 0.125
1000	92.63 ± 0.111	93.34 ± 0.071	93.60 ± 0.216	93.68 ± 0.106	94.01 ± 0.115
IC 50 Values	478.446	454.362	428.664	420.569	447.972

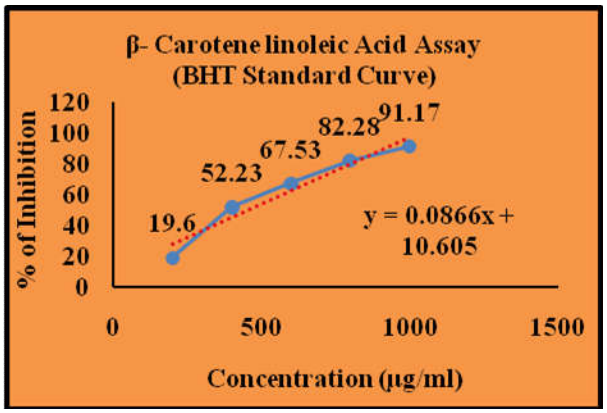

Figure 21. β carotene linoleic acid assay BHT standard curve

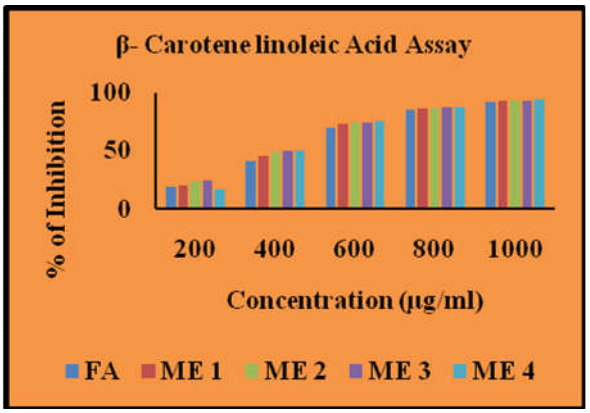

Figure 22. β carotene linoleic acid assay of encapsulated and non-encapsulated astaxanthin

DISCUSSION

An antioxidant is a compound which counteracts the effect of oxidized and controls the build of free radicals. Due to the presence of different antioxidant components in the samples, it is relatively difficult to measure each antioxidant component separately. Therefore, several assay methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such samples (K. R. Prabhakar *et al,* 2006; H. Wangensteen *et al,* 2004). These methods target at different mechanisms of the oxidant defense system such as, scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions (Pandithurai and S Murugesan, 2014). Carotenoids are components that play an important role in biological systems, starting with light protection, immuno enhancement, protection against carcinogens and finishing with antioxidant activity (H. Kurihara *et al,* 2002; Dufosse *et al,* 2005; Sikora *et al,* 2009). There are many carotenoids widely applied in feed, pharmaceutical, food and cosmetics industry (Fraser and Bramley, 2004). The world market of food additives is based mainly on synthetic additives; however, higher consumer awareness has resulted in an increased use of natural substances (J. Pokorny, 2007). One of the new potentially antioxidant compounds could be a lipid soluble carotenoid – astaxanthin (xanthophyll) (H. Jackson *et al,* 2008). In present study, antioxidant activity of encapsulated and non-encapsulated astaxanthin was done by different types of methods. From all the result it is founded that most of the test shows higher antioxidant than the standard drugs. The highest percentage was obtained for ME 4 sample along with non-encapsulated astaxanthin.

The present study was compared with other research articles. J. G. Bell *et al,* 2000 studied was carried out with salmon which showed that the antioxidant synergism of vitamin E and astaxanthin reduced malondialdehyde formation in an *in vitro* stimulation of microsomal lipid peroxidation. Oxygen derived free radicals or reactive oxygen species (ROS) formed in the body during energy producing metabolic process, play an important role in pathophysiology of a number of diseases (S. Cuzzocrea *et al,* 2001). Normally oxygen free radicals are neutralized by natural antioxidants. However, ROS become a problem when either a decrease in their removal or their overproduction occurs, resulting in oxidative stress. This stress and the resultant damage have been implicated in many diseases and a wealth of preventive drugs and treatments are currently being studied. Thus, astaxanthin exhibiting multiple antioxidant activity will find utility in applications like antioxidant therapy, which is based on reducing oxidative stress in the target tissues. Since synthetic astaxanthin is a mixture of three stereoisomers (3R, 3'R; 3S 3'S; 3R, 3'S) astaxanthin from natural sources is preferred for using it as an antioxidant. Astaxanthin from natural sources is abundant in the isomer showing highest biological activity (3R, 3'R; 3S 3'S). B. S. Kamath *et al,* 2008 has reported that the IC50 values for free radical scavenging activity of *Haematococcus pluvialis* astaxanthin esters *in vitro* were 8.0 µg /ml. S. Sindhu and P. M. Sherief, 2011 research reveals that, astaxanthin extracted from shell waste of *Aristeus alcocki* possessed significant hydroxyl radical scavenging activity, lipid peroxidation-inhibiting activities and superoxide radical-scavenging activity.

The extract showed 50% inhibition (50 % inhibiting concentration) at concentrations 56.43 ± 1.06 ng/ml, 26.54 ± 0.42 ng/ml, and 27.91 ± 0.54 ng/ml. The standard antioxidants quercetin and catechin showed antioxidant activity at microgram levels whereas astaxanthin present in shrimp shell extract showed *in vitro* antioxidant activity at nano gram levels. This clearly indicates the high antioxidant potential of astaxanthin extracted from *Aristeus alcocki* shell waste. Aline Alves Barbosa – Silveira *et al,* 2015 study clearly demonstrates the scavenging action of the DPPH free radical and this is very important information for astaxanthin studies. The DPPH free radicals were decreased by approximately 10% at 1 mg/ml of astaxanthin; by 50% at 10 mg/ml and by about 80% at 100 mg/ml of astaxanthin in all conditions that were tested and compared to the control. Shengzhao Dong *et al,* 2014 research indicated that scavenging activities and reducing power of extract obtained by HCl- ACE extraction method were the highest, respectively. While scavenging activities and reducing power of extract obtained by oil-soy extraction method were the lowest, the reasonable explanation was probably that the astaxanthin content in extract obtained by HCl-ACE was the highest, while the astaxanthin content in extract obtained by oil-soy was the lowest. It suggested that astaxanthin was the prominent factor for the antioxidant character of extracts obtained by four extraction methods. Thus, our present study showed that, the antioxidant effect of astaxanthin content agreed with the previous reports.

Conclusion

The results of the present study suggested that both free Astaxanthin and encapsulated Astaxanthin exhibits good antioxidant and radical scavenging properties. The quenching effect on free radicals and lipid peroxidation varies between encapsulated Astaxanthin synthesized and characterized by different methods. Thus, Astaxanthin in esterified form can be used as potent drug for various diseases associated with cellular damage and oxidative stress. Further, it could also be explored for anti-inflammatory and other pharmacological properties.

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