International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

COMPARITIVE STUDY OF ANTIOXIDANT ACTIVITY AND INHIBITION TO CHOLESTEROL OXIDATION OF *ZINGIBER OFFICINALE* AND *APIUM GRAVEOLENS*

Banani De* and Suchanda Chatterjee

School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah – 711 103, India.

ABSTRACT

Cholesterol oxidation products, which are more injurious to adult human than pure cholesterol, may occur in vivo or during food processing through cholesterol autoxidation. Also current interest in natural products has stimulated the search for new cholesterol-lowering agents from herbal sources. The aim of this study is to estimate the antioxidant activity of two common spices -dry ginger (*Zingiber officinale*) and celery seeds (*Apium graveolens*) and to determine their efficacy in controlling the cholesterol oxidation in fish lipid both in presence of enzyme and under non-enzymatic storage condition. Antioxidant activity was evaluated from chloroform extract of these spices in terms of total phenol content, ferric reducing activity, inhibition to peroxidation in linoleic acid system and metal chelating activity over a span of five weeks, and compared against synthetic antioxidant-BHT (butylhydroxytoluene). Total cholesterol was estimated from tilapia fish lipid extracted post refrigerated preservation (0-4^{0}C) of spiced, homogenized fish muscle, at interval of one week on day 0, 7, 14, 21, 28 and 35. Phenol content and ferric reducing activity was found to be higher in ginger. In terms of phenol content, inhibition to peroxidation in linoleic acid and metal chelating activity, compared to ginger, antioxidation potential was found to increase in celery after first two weeks. Though both of them could comparably inhibit cholesterol oxidation ginger showed its maximum efficiency till first three weeks while celery till day 14, but in long run celery seeds were found to be more efficient in controlling cholesterol oxidation product formation. Synthetic antioxidant BHT recorded a lower antioxidant potential but a higher efficiency in controlling cholesterol oxidation.

Keywords: Ginger, Celery Seeds, Antioxidant, Cholesterol oxidation, Fish lipid.

INTRODUCTION

Cholesterol, a monounsaturated lipid with a double bond on carbon-5, is a relatively stable compound and is only susceptible to oxidation under harsh conditions in presence of oxygen, light, heat, radiation, free radicals, metal ions, and other factors [1]. Cholesterol oxidation products (COPs) are group of sterols structurally analogous to cholesterol but contain an additional hydroxy, ketone or epoxide group on the sterol nucleus and/or a hydroxyl group on the side chain of their molecules. They may also arise in vivo or during food processing through cholesterol autoxidation which involves several chemical reactions of cholesterol with free radicals. Peroxidation of cholesterol by singlet oxygen produces primarily a C-5 oxygenated molecule: 5α-hydroperoxycholesterol [2]. This molecule may be later rearranged giving 7α-hydroperoxycholesterol which is progressively epimerized into 7β-hydroperoxycholesterol. The most important autoxidation products are 7-keto and 7β-OH cholesterol [3]. 7α-OH or 7β-OH cholesterol are further transformed into hydroxylated derivatives or into 7-keto cholesterol, C-5 and C-6 oxygenated derivatives**,** i.e., 5,6α- or 5,6β-epoxides which are further transformed into a triol derivative (Kumar and Singhal, 1992).Further complications arise from the possible in vivo formation of

Corresponding Author: - **Banani De** Email: bosebanani@gmail.com

some other oxysterols by enzymatic reaction. These reactions mainly form oxygenated derivatives in C-7 position and other products result due to subsequent reactions on the lateral chain at C-20, C-22, C-24, C-25 and C-27 [3].

Cholesterol oxidation products as well as fatty acid oxidation in food system have been a concern for adult human health. Cholesterol oxidation products (COPs) are known to be more injurious to arterial cells than pure cholesterol and are more directly connected to the development of atherosclerosis and coronary heart disease [1,4]. COPs deteriorate the bioavailability of cholesterol by inhibiting cholesterol biosynthesis [5] and dietary uptake of cholesterol [6]. COPs also impair a membrane function, which results in altered membrane permeability [7]. On the other hand, natural cholesterol had no atherogenic or hypercholesterolemic effect and has a much lower influence on the activities of most enzymes than COPs.

24S-Hydroxycholesterol (cerebrosterol) is an enzymatically oxidized product of cholesterol mainly synthesized in the brain [8]. An overview of several studies on cerebrosterol has suggested its possible connection with neurodegenerative diseases [9]. It must be noted that 24S-hydroxycholesterol was found in high concentration in atheroma plates. Cholestanetriol and 25-hydroxycholesterol, secondary COPs, which can be derived from primary COPs, were reported to be the most atherogenic among oxysterols studied [10,6] and caused remarkably acute injury to the endothelium of rabbits. It is well known that these compounds display cytotoxic, pro-apoptotic, and pro-inflammatory activities. Several investigations suggest that they act as endogenous regulators of gene expression in lipid metabolism and as cell signaling molecules [11]. Numerous studies show that oxysterols are associated with various types of cancer [12].

Current interest in natural products has stimulated the search for new cholesterol-lowering agents from these sources. Many herbal medicinal products were reported to have a potential to reduce lipid and cholesterol in body and to enhance the safety profile. Oxidative modification of LDL cholesterol is thought to play a key role during atherosclerosis.

Plants contain a variety of antioxidants (including vitamins C and E and the carotenoids) that can markedly inhibit oxidation of LDL cholesterol via a mechanism involving scavenging of free radicals [13]. Ethanolic ginger extract showed an ability of lowering liver cholesterol and cholesterol oxidation in E0 rats [14]. *A. graveolens* extracts have different beneficial biological activities as is reported by Jiao *et al*., (2003) [15]. The isolated compounds from the seeds exhibited antioxidant and inhibitory effects of cyclooxygenase and topoisomerase enzymes (type I and II) [16]. *Apium graveolens* L. has shown good antihyperlipidemic effect and could be of value in reducing serum total cholesterol, triglycerides, LDL-c and increasing HDL-c [17]. The aim of this study is to estimate the antioxidant activity of these two spices ginger and celery seeds and to determine the efficacy of these two herbs in controlling the cholesterol oxidation in fish lipid both in presence of enzyme and under non-enzymatic storage condition.

MATERIAL AND METHODOLOGY

Chemicals

2-Thiobarbituric acid was purchased from Loba chemie (India). Ammonium thiocyanate and ferric chloride, choloroform, methanol, isooctane all other solvents and reagents were procured from Merck (India). All chemicals used were of analytical grade.

Spices

Dry ginger (*Zingiber officinale*) and celery seeds (*Apium graveolens*) were bought from local market in south Kolkata and grinded and sieved at125 micron.

Preparation of fish sample

Total amount of 3 Kg of Tilapia fish (each having an average weight of 300 gram, length 6.5 inches and age of 3 months) was procured from Chowbaga bheri located in east Kolkata wetland. Fish muscles were minced and homogenized in a blender. The mass was further divided in 21 (4x5 + 1) equal portions each weighing 100 gms for mixing the two spices, a synthetic antioxidant BHT and one group as non-spiced control. Thus four groups were made. Both natural and synthetic antioxidant, powdered dry ginger and celery seeds having particle size 125 micron and BHT were used at a proportion of 10% by weight of the sample (ie. 10 gm spice powder was used for each of 100 gm of fish sample) and were put in the press-and-lock polythene freezer bag (49 micron thickness) and stored in the freezer chamber of a refrigerator at 0-4 ^{o}C. Four groups of samples were kept in five batches and taken out for analyses at day 0, 7, 14, 21, 28 and 35. The experiment was repeated thrice.

ESTIMATION OF ANTIOXIDANT ACTIVITY IN SPICES

Sample preparation for spices

An amount of 5 gm of spice sample was extracted with 50 ml chloroform. It was further treated with activated charcoal to decolourize, then centrifuged and filtered with Whatman 1 filter paper. This solution was used for execution of all the antioxidant tests mentioned.

Sample preparation of fish lipid

The fish oil extracted following Bligh Dyer method from various spiced samples and non-spiced control on day 0, 7, 14, 21, 28 and 35 was taken in chloroform and the antioxidant tests were performed following the stated standard protocol.

Extraction of lipid

It was done by Bligh and Dyer method (1959). Homogenization of 100gm of blended fishes was done with 1:1 methanolic chloroform and the filtered through Whatman no. 1 filter paper. The organic layer were separated through separatory funnel and dried with anhydrous sodium sulphate. The solvent was removed at low temperature (40 ^{o}C)

Weight of lipid = (weight of container + extracted lipid) - (weight of container)

Lipid content (%) = amount of lipid extracted (g)/weight of original sample (g) X 100

These lipids were used to estimate the total cholesterol content.

Determination of total phenolic compounds

Total phenolic compound was determined using Folin-Ciocalteu reagent using the modified method of Wolfe *et al*., (2003). A sample solution of 0.2 mL was pipetted in glass tube and 1 ml of Folin-Ciocalteu reagent, 0.8 ml of sodium carbonate (7.5%) was added to it. The mixture was stored at room temperature for 30 min and absorbance was recorded at 765 nm. Total phenolic compounds were calculated using a standard curve prepared with dilutions of gallic acid. Gallic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE) /g of extract.

Iron(III) to iron(II)-reducing activity

The reductive potential of the extract was determined according to the method of Oyaizu (1986). Different concentrations of extracts and standard (0.2, 0.4, 0.6, 0.8, 1.0 mg mL^{-1}) in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v) and the absorbance was measured at 700 nm in a spectrophotometer. Higher the absorbance of the reaction mixture indicated greater reductive potential. Tannic acid was considered as standard. The experiment was carried out in triplicate set.

Antioxidant activity in a linoleic acid system

Antioxidant activity was evaluated by the thiocyanate method [18]. Sample was added to 0.5 ml of chloroform, linoleic acid emulsion (2.5 mL 0.02 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0) in a test tube and stored in darkness, at 37 ^{o}C, to accelerate oxidation. The linoleic acid emulsion was prepared by mixing an equivalent weight of linoleic acid and Tween 20 in phosphate buffer (0.2 M, pH 7.0). The peroxide value was determined by reading the absorption at 500 nm with a spectrophotometer, after colour development with $FeCl_2$ and thiocyanate at various intervals during incubation. The peroxidation of linoleic acid was calculated as peroxidation (%) = (A^1/ A^0) X 100,

where A^0 = the absorption of the control reaction and

A^1 = the absorption in the presence of sample.

Metal chelating activity

The chelation of ferrous ions by the extracts and standard was estimated by the method of Dinis *et al*. (1994). Extracts were added in different concentrations (0.2 to 1 mg mL^{-1}) to a solution of 1mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 1 mM Ferrozine (0.1 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were done in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below [19].

$$PI = \frac{A_{(control)} - A_{(sample\ or\ standard)}}{A_{(control)}} \times 100$$

A(control) = Absorbance of control reaction

A(sample or standard) = Absorbance of sample or standard

Estimation of cholesterol in fish lipid

Cholesterol was estimated by CHOD – PAP method. It is an enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine.

In this method the blank contained only 1 ml of working reagent. The standard contained 1ml reagent and 0.01 ml cholesterol standard and the specimen contained 1 ml reagent and 0.01 mL test sample. After 5 minutes of incubation at 37 ^{0}C the absorbance of standard (Abs S) and specimen (Abs T) was measured at 505 nm and compared against reagent blank. The amount of cholesterol was calculated by using this formula

Cholesterol in mg/dl= Abs T/Abs S * 200

Statistical analysis

The experiment was performed in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P < 0.05$). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 16.0 for windows, SPSS Inc.).

RESULTS AND DISCUSSION

Estimation of antioxidant activity in spices

Total phenol content

Phenolic compounds are considered to be important plant materials because of their inhibitory effect on autoxidation of lipids [20] and their radical scavenging ability [21]. Therefore, it is important to determine the total phenolic compound in the spices. From Figure. 1 it is

explicit that highest phenolic content was recorded in ginger followed by celery and BHT respectively. It is reported that rhizome of ginger contains over twenty phenolic compounds of which the important ones are gingerols [22], shogaol and diarylheptanoids [23] whereas twenty-nine phenolic compounds were isolated from the root bark of fresh (Yunnan) ginger [24]. Celery belonging to a part of the Umbelliferae plant family, is also rich in phenolics like hydroxycoumarins (apigravin, celerin, osthenol), isoquercitrin, and umbelliferone [25]. A gradual and significant ($P< 0.05$) drop in the phenolic content with time was observed in both ginger and BHT whereas phenolic content of celery increased after day 14^{th} and gradually maintained linearity in slope for the rest of the period. Increase in concentration might be due to the fact that polyphenolic compounds are usually present as glycosides in plant sources and hence might undergo hydrolysis with time under suitable condition to release the flavour molecules [26].

Iron(III) to iron(II)-reducing activity

Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [27]. The iron (III) to iron (II)-reducing activity is expressed as tannic acid equivalents (mmol tannic acid/g sample). Figure.2 shows that the best activities were found for ginger. Celery and BHT delineates a comparable ferric reducing activity. All the additives had undergone a significant ($P<0.05$) fall in their reducing potential with time. Celery recorded an initial increase ($P<0.05$) till day 14 which was followed by a significant ($P<0.05$) drop in the value after that. BHT maintained a gradual decline in the activity with time.

Antioxidant activity in linoleic acid emulsion

Linoleic acid being a polyunsaturated fatty acid easily undergoes autooxidation in air leading to occurrence of coupled double bonds, and subsequently secondary products, such as aldehydes, ketones, and alcohols as a result of series of reaction (Dobarganes and Velasco, 2002). Spices being natural antioxidants are expected to inhibit the peroxidation. The graph (Figure. 3) expresses the extent of peroxidation in linoleic acid system with addition of spices. The lower peroxidation values in linoleic acid indicate higher inhibition to peroxidation of these spices. Comparatively higher value in case of BHT than celery and ginger respectively indicates that higher oxidation is occurring in linoleic acid system when BHT is used. This implies that BHT could not inhibit the extent of peroxidation effectively than celery and ginger. Peroxidation in linoleic acid system had been most successfully controlled when ginger is added to the system. Hence the antioxidant activity order can be expressed as ginger > celery > BHT. It is explicit from Figure.3 that both celery and BHT had shown a significant ($P < 0.05$) rise in the level of oxidation with time till day 14 and then has undergone a decrease in the subsequent weeks ($P < 0.05$). On the contrary instead of reduction the peroxidation has shown a sharp increase in value from 21^{st} day. This indicates significant ($P < 0.05$) increase in antioxidant activity of BHT and celery from day 14 and fall in antioxidant potential of ginger with longer time duration. With regard to gingerol and/or shogaol it is estimated that for short-term storage at least, gingerol and shogaol are relatively stable in the absence of a catalyst such as strong acids or bases [28]. It can be thus inferred that though ginger is more efficient antioxidant in the initial days or for a shorter time span, celery followed by BHT is more effective antioxidant in long run. This change in the antioxidant activity in case of celery and ginger after day 14, perfectly corroborates with the increasing and decreasing phenolic content respectively (Figure.1).

METAL CHELATING ACTIVITY

Ferrozine can quantitatively form complexes with Fe^{2+}. However, in presence of other chelating agents this complex formation is disrupted. This results in the decrease in red colour intensity of the solution. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The ferrous ions possess the ability to move single electrons by virtue of which it triggers propagation of many radical reactions, even with relatively non-reactive radicals [29]. Main strategy to avoid the reactive oxygen species generation is through chelating metal ions. Figure. 4 exhibits comparable metal chelating activity for both celery and ginger followed by BHT. Metal chelating activity of celery and ginger had increased steeply after the first week and then followed a decrease in the subsequent week. Celery had again increased slightly on day 21^{st} and maintained its linearity thereafter. After day 7 metal chelating activity of ginger declined gradually ($P < 0.05$).

Cholesterol estimation

The series of reactions involved in the assay system are as follows:

1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids. (Equation 1)
2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and H_2O_2. (Equation 2)
3. In presence of peroxidase (POD), the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye. (Equation 3)

Cholesterol esters+H_2O = Cholesterol + fatty acids …(1)

Cholesterol +O_2 = Cholest-4-ene-3-one+H_2O_2 …….(2)

2 H_2O_2+ 4-AAP + Phenol = Quinoneimine dye + 4 H_2O……..(3)

The above equations indicate that cholesterol is measured from the amount of hydrogen peroxide generated due to oxidation of cholesterol to its oxide. The hydrogen peroxide is basically generated from two sources, one is due to enzymatic oxidation as a result of addition of peroxidase and secondly due to non-enzymatic lipid oxidation occurring due to storage. Cholesterol concentration of the fish oil extracted from refrigerated spiced fish samples and control recorded over a span of five weeks is shown in Figure. 5. It is observed that cholesterol concentration in control (containing no spices) increased significantly (P<0.05) with an uniformity during this period and a remarkable rise is observed after day 14. Cholesterol being a monounsaturated lipid is susceptible to oxidation and hence undergoes autoxidation to form cholesterol oxide product. Smith (1987) also suggested that the hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation initiates cholesterol oxidation. The degree of oxide formation is related to storage conditions and level of activator present. Therefore it is conceivable, that cholesterol oxidation should proceed in a way analogous to fatty acid oxidation. Since total cholesterol concentration cannot change during storage period, the increasing value of cholesterol recorded in control reflects the non-enzymatic oxidation of cholesterol which accumulated considerable amount of hydrogen peroxide with lipid oxidation. This peroxide developed the red colour with phenol and 4-AAP.

Addition of spices recorded lower concentration of cholesterol in all the cases. Presence of spice additives (ginger, celery, BHT) scavenge the peroxide generated due to enzymatic oxidation thereby resulting in reduction of the cholesterol values of the spiced fish oil. Though values in celery, ginger and BHT added fish lipid has shown a diminishing trend initially (P<0.05) ultimately followed an increment (P<0.05) after 14th, 21st and 28th day respectively. This increasing gradient in the later period of storage is due to hydrogen peroxide generated due to non-enzymatic oxidation occurring during storage. Ginger and celery was found to exhibit a comparable antioxidant activity to control the oxidation of cholesterol, whereas BHT was more efficient in this regard. Ginger and celery recorded the lowest concentration of 4.46 mg/dl and 5.37 mg/dl on 21st and 14th day respectively whereas BHT exhibited the minimum of 2.36 mg/dl on 28th. Though there is significant overall decrease in value due to addition of spices, both celery and ginger could not completely control oxidation till the end. It should be noted that till the last day only BHT could maintain a value lower than 1st day and decreased the concentration uniformly till the end (P<0.05).

The above findings indicates that possibly both celery and ginger can remarkably control the cholesterol oxidation (P<0.05) which arrests the formation of COPs in animal products.

CORRELATION STUDY

Correlation between the parameters of antioxidant activities like phenol content, peroxidation in linoleic acid, metal chelating and ferric reducing activity were determined. Phenol content has shown a good correlation with peroxidation in linoleic acid and ferric reducing activity in case of all the three additives. Ginger exhibited high positive correlation (0.869867) in phenol content & ferric reducing activity whereas has negatively correlated in phenol content & peroxidation in linoleic acid and ferric reducing activity & peroxidation in linoleic acid (-0.83348 and -0.95889 respectively). Phenol content of celery has negatively correlated with ferric reducing activity and peroxidation in linoleic acid (-0.78857, -0.57895 respectively) and showed a high positive correlation (0.930516) in ferric reducing activity & peroxidation in linoleic acid system. Correlation, though not high was found to be over all positive for metal chelating activity and peroxidation in linoleic acid system (Table1).

Figure 1. Total phenol content of spices (GAE; mg gallic acid /g of extract)

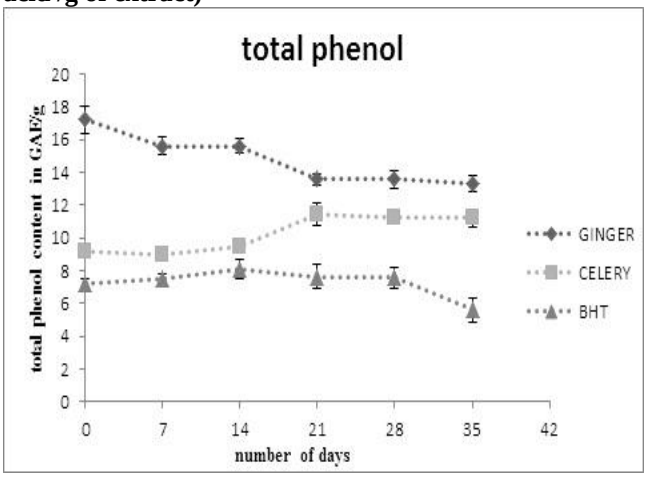

Figure 2. Ferric reducing activities of spices (TAE; mg Tannic acid /g of extract)

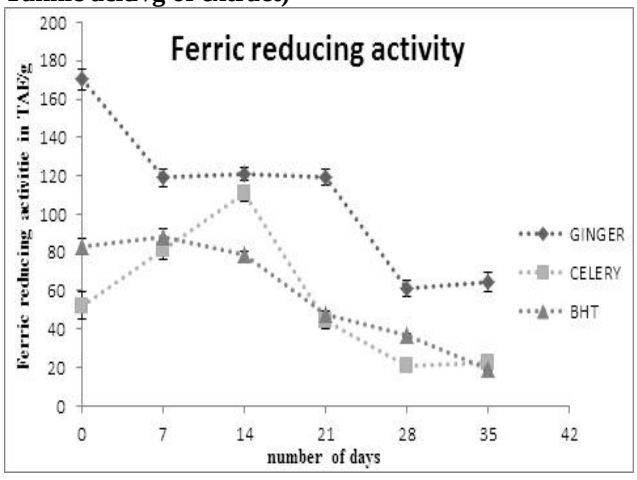

Figure 3. Percentage of oxidation in linoleic acid system on addition of spices

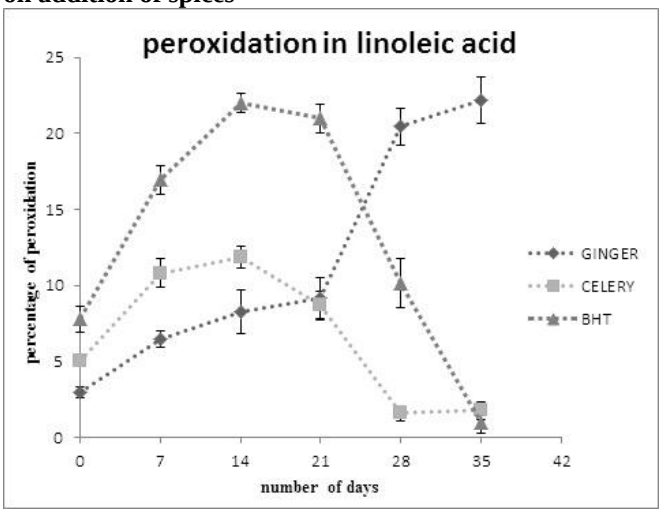

Figure 4. Metal chelating activities of the spices

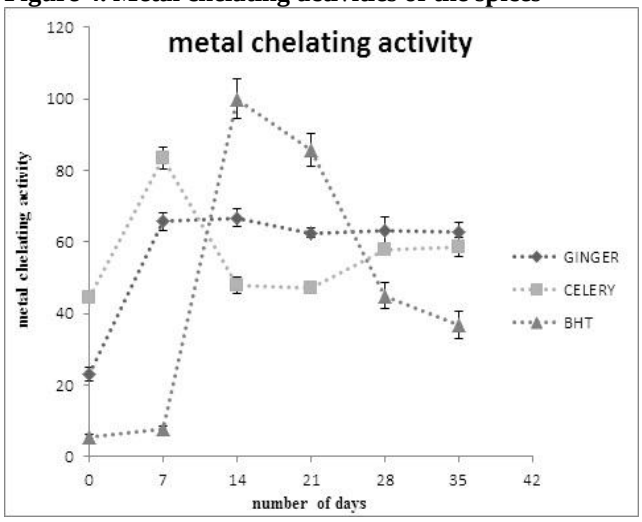

Figure 5. Cholesterol content of the lipid after addition of spice additives

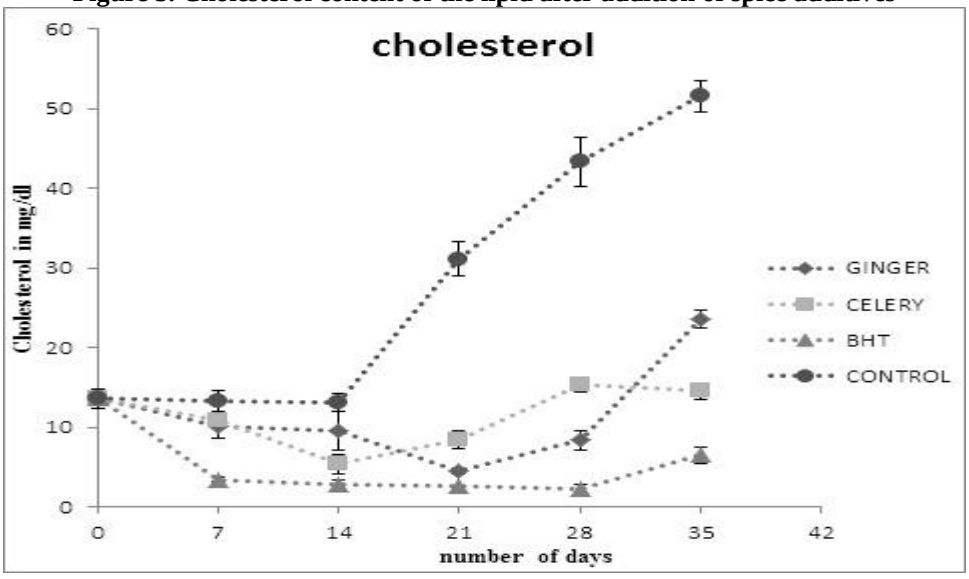

Table 1. Correlation study of the antioxidant parameters

Ginger	Celery	BHT
phenol content: peroxidation in linoleic acid		
-0.83348	-0.57895	0.86253
peroxidation : metal chelating		
0.485135	0.155132	0.604112
phenol content: ferric reducing activity		
0.869867	-0.78857	0.578347
ferric reducing activity : peroxidation		
-0.95889	0.930516	0.543236

CONCLUSION

This study has provided us with some interesting and important findings. It clearly indicates the comparable and high antioxidant activity of both celery and ginger which is almost equivalent to commonly used synthetic antioxidant BHT. Both celery and ginger can remarkably arrests the formation of cholesterol oxidized products (COPs) in animal products. Contrary to ginger, celery was found to enhance its antioxidation potential after first two weeks in terms of phenol content, inhibition of peroxidation of linoleic acid and metal chelating activity. Celery could control cholesterol oxidation with maximum

efficiency till day [14] whereas ginger could do so till day [21]. In long run celery was found to be more effective since antioxidant potential of ginger reduced markedly after day [28].

ACKNOWLWDGEMENT

This study was financially supported by J.D.Birla Institute. Kolkata, India.

REFERENCES

1. Paniangvait P, King AJ, Jones AD, German BG. Cholesterol oxides in foods of animal origin. *Journal of Food Science*, 60(6), 1995, 1159–1174.
2. Maerker G. Cholesterol autoxidation–current status. *Journal of American Oil Chemists Society*, 64(3), 1987, 388–392.
3. Park SW, Addis PB. Identification and quantitative estimation of oxidized cholesterol derivatives in heated tallow. *Journal of Agricultural and Food Chemistry*, 34(4), 1986, 653–659.
4. Addis PB. Occurrence of lipid oxidation products in foods. *Food Chemical Toxicology*, 24(10/11), 1986, 1021–1030.
5. Lund E, Bjorkhem I. Down-regulation of hepatic HMG CoA reductase in mice by dietary cholesterol: importance of the 5- double bond and evidence that oxidation at C-3, C-5, C-6 or C-7 is not involved. Biochemistry, 33(1), 1994, 291–297.
6. Peng SK, Taylor CB, Hill JC, Morin RJ. Cholesterol oxidation derivatives and arterial endothelial damage. *Atherosclerosis*, 54(2), 1985, 121–133.
7. Hennig B, Boissonneault GA. Cholestan-3b, 5a, 6b-triol decrease barrier function of cultured endothelial cell monolayers. *Atherosclerosis*, 68(3), 1987, 255–261.
8. Ercoli A, De Ruggieri P. The constitution of cerebrosterol, a hydroxycholesterol isolated from horse brain. *Journal of the American Chemical* ***Society***, 75(13), 1953, 3284-3284.
9. Björkhem I. Rediscovery of cerebrosterol. *Lipids*, 42(1), 2007, 5-14.
10. Taylor CB, Peng SK, Werthessen NT, Tham P, Lee KT. Spontaneous occurring angiotoxic derivatives of cholesterol. *American Journal of Clinical Nutrition*, 32(1), 1979, 40–57.
11. Olkkonen VM. New Functions for Oxysterols and Their Cellular Receptors. *Lipids Insights*, 2, 2008, 1-9.
12. Jusakul A, Yongvanit P, Loilome W, Namwat N, Kuver R. Mechanisms of oxysterol-induced carcinogenesis. *Lipids in Health and Disease*, 10, 2011, 44.
13. Fuhrman B, Buch S, Vaya J, Belinky PA, Coleman R, Hayek T, Aviram M. Licorice extract and its major polyphenol glabridin protect low-density lipoprotein against lipid peroxidation: in vitro and ex vivo studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *American Journal of Clinical Nutrition*, 66(2), 1997, 267–275.
14. Fuhrman B, Rosenblant M, Hayek T, Coleman R, Aviram M. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic lipoprotein E-deficient mice. *Journal of Nutrition*, 130(5), 2000, 1124–1131.
15. Jiao XZ, Xie P, Zu LS, Liang XT. Study of stereoselective synthesis of (+/-) Neocnidilide. *Journal of Asian Natural Products Research*, 5(3), 2003, 165-169.
16. Momin RA, Nair MG. Antioxidant, cyclooxygenase and topoisomerase inhibitory compounds form *A. graveolens* seeds. *Phytomedicine*, 9(4), 2002, 312-318.
17. Mansi K, Abushoffa AM, Disi A, Aburjai T. Hypolipidemic Effects of Seed Extract of Celery (*Apium graveolens*) in Rats. *Pharmacognosy Magazine*, 5(20), 2009, 301-305.
18. Mitsuda H, Yasumoto K, Iwami K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo*, 19(3), 1966, 210–214.
19. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry Biophysics*, 315(1), 1994, 161-169.
20. Ramarathnam N, Osawa T, Namiki M, Tashiro T. Studies on the relationship between antioxidative activity of rice hull and germination ability of rice seed. *Journal of the Science of Food and Agriculture*, 37(8), 1986, 719– 726.
21. Hatano T, Edamatsu R, Hiramatsu M, Mori A. Effect of interaction of tannins with co-existing substances VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chemical and Pharmaceutical Bulletin*, 37(8), 1989, 2016-2021.
22. Shoji N, Iwasa A, Takemoto T, Ishida Y, Ohizumi Y. Cardiotonic principles of ginger (*Zingiber officinale* Roscoe). *Journal of Pharmaceutical Sciences*, 71(10), 1982, 1174–1175.
23. Kikuzaki H, Kobayashi M, Nakatani N. Diarylheptanoids from rhizomes of *Zingiber officinale***.** *Phytochemistry*, 30(11), 1991, 3647–3651.
24. Peng F, Tao Q, Wu X, Dou H, Spencer S, Mang C, Xu L, Sun L, Zhao Y, Li H, Zeng S, Liu G, Hao X. Cytotoxic, cytoprotective and antioxidant effects of isolated phenolic compounds from fresh ginger. *Fitoterapia*, 83(3), 2012, 568-585.

25. Lu ZG, Li W and Wang PJ. Chemical Composition and Ability of Scavenging DPPH Radical of Essential Oil and Residue from the Celery Seed. *Advanced Material Research,* 2011, 183-185, 18-21.
26. Corradini E, Foglia P, Giansanti P, Gubbiotti R, Samperi R, Lagana A. Flavonoids: chemical properties and analytical methodologies of identification and quantitation in foods and plants. *Natural Product Research,* 25(5), 2011, 469-495.
27. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food Chemistry,* 49(8), 2001, 4083-4089.
28. Bhattarai S, Tran VH, Duke CC. The Stability of Gingerol and Shogaol in Aqueous Solutions. *Journal of Pharmaceutical Sciences*, 90(10), 2001, 1658-1664.
29. Aboul-Enein AM, El-Baz FK, El-Baroty GS, Youssef AM, Abd El-Baky HH. Antioxidant activity of algal extracts on lipid peroxidation. *Journal of Medical Sciences*, 3(1), 2003, 87-98.
30. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 1959, 911–917.
31. Dobarganes MC, Velasco J. Analysis of lipid hydroperoxides. *European Journal of Lipid Science and Technology*, 104(7), 2002, 420–428.
32. Kumar N, Singhal OP. Effect of processing conditions on the oxidation of cholesterol in ghee. *Journal of Science of Food and Agriculture*, 58(2), 1992, 267–273.
33. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *Journal of Agricultural Food Chemistry,* 51(3), 2003, 609-614.