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EVALUATION OF ANTIOXIDANT ACTIVITIES OF PLANT EXTRACTS ENCAPSULATED IN LIPOSOMES

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ABSTRACT

The antioxidant activity of methanolic extracts of *Cuminum cyminum* L. (cumin) and *Garcinia cambogia* L. (cambodge) and an antipeptide Nisin was determined. The different methods like DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), H_2O_2 (Hydrogen peroxide), Hydroxyl radical scavenging activity, Nitric oxide scavenging assay, and Reducing power capacity were measured with before and after encapsulation of phosphatidyl choline liposomes. All the samples showed dose dependent increase in antioxidant activity. The higher concentration at 50 µg/mL indicated much superior level of antioxidant properties and after the encapsulation in liposomes such as Liposome Cumin (LC), Liposome Cambodge (LCa) and Liposome Nisin (LN) showed increased level of antioxidant activity than the other three samples Cumin, Cambodge and Nisin (C, Ca, N) in original form. Butylated hydroxy toluene (BHT), used as control, resulted in the highest percentage of inhibition in all the methods. All the samples showed better inhibition or scavenging activity in every antioxidant method and the results were expressed as significant at $p < 0.05$.

Key-words: Antioxidant activity, *Cuminum cyminum*, *Garcinia cambogia*, DPPH, ABTS, H_2O_2, Nitric oxide, Reducing power capacity, Hydroxyl scavenging activity, Phosphatidyl choline liposome, BHT

INTRODUCTION

Garcinia cambogia L. (Family-*Guttiferae*, also known as Cambodge) is a traditionally used medicinal plant in India. The fruit of *Garcinia* is commonly used as a food preservative, and also have various antimicrobial properties (Roy *et al*., 2003). *Garcinia* fruit also known as "Malabar Tamarind" has sour taste and dark in colour and which has been used as a main ingredient in fish curries in Southern Asian countries. Also, Malabar tamarind is much effective in reducing body weight and bad cholesterol. A study with citric acid, (-) Hydroxycitric acid indicated some obesity related trials in rats. After consumption of dose of 3mg/kg of this acid rats showed decreased eating tendency and carbohydrate cravings. This is a main acid in *Garcinia cambogia* L. (Sergio *et al*., 1988).

Cuminum cyminum L. *(cumin)*, belonging to the family *Apiaceae*, is widely used spice in India, Middle East etc. The fruit (seed) have aromatic strong smell and also contributes various medicinal principles. Seeds are mainly used for main additives in foods. Cumin is most popular in Ayuerveda, mainly for gastric problems. Some evidences are there that cumin seeds (aqueous extract) can reduce the blood sugar, plasma and lipid levels, also prevent excessive weight gain in rats (Dhandapani *et al*., 2002).

Nisin coming under the class I bacteriocin, a protein, cannot be digested by digestive enzymes. World Health Organisation (WHO) announced Nisin as a safe food preservative with no potential side effects. Currently, Nisin is used as a food preservative commercially. Liu *et al*. (2020) proved that the fermentation products of Nisin and GABA (γ-aminobutyric acid) are very good antioxidants and possess antimicrobial properties. Due to the physiological functions, GABA has been recognised as a food preservative in Japan approved by Food Drug Administration. Nisin is functionally active against Gram positive bacteria but not in case of against Gram negative bacteria. Nisin have been reported to have many antimicrobial activities (Punyauppa-path *et al*., 2015). Nisin shows antibacterial activities mainly against food spoilage bacteria (Hansen *et al*., 1994). Research showed that Nisin has antitumor potential and also inhibit growth of Gram-negative bacteria. WHO has recommended that consumption of 0.6mg of Nisin a day is safe and normal (Joo *et al*., 2012).

Liposomes are colloidal, vesicular structures based on (phospho) lipid bilayers. In these structures, an aqueous core is surrounded by lipids arranged in a bilayers configuration. They can be as small as 20 nm and as large as several microns in diameter (Torchilin, 2005). Liposomes are considered as an alternative drug carrier system because of their easy preparation (Chen *et al*., 2012) and also innovative technology in Pharma industries for drug delivery. Liposomes are mainly composed of phosphatidyl choline. Addition of long chain amine results positively charged liposomes. Neutral liposomes consist of cholesterol and phospholipids. The main benefits of liposomal drug delivery results low dosage, low allergic reactions and easy cellular permeability, etc. (Fendler *et al*., 1977).

Nowadays, synthetically prepared antioxidants are of great concern in pharmaceutical industries and food industries because of their harmful side effects. There is scarcity of natural antioxidants with low toxicity with no side effects. The easily available natural antioxidant sources are plants, animals and microorganisms, which are nutritionally rich but very expensive. To overcome, the expensiveness of such products, scientists are focusing on research to produce low cost methods for the production of natural antioxidant products especially from herbal source (Sayyed-Alangi *et al*., 2019). Therefore, in this study we focused on different antioxidant methods of *Cuminum cyminum Garcinia cambogia* and Nisin. We aimed to find out, the encapsulated phosphatidyl choline liposomes with their pure samples.

MATERIALS AND METHODS

Sample collection and preparation

The samples such of *Cuminum cyminum* L. and *Garcinia cambogia* L. were collected from Spices Board Cochin, Kerala, India. Nisin was purchased from HiMedia Laboratories, LLC, USA. The samples were dried in laminar chamber hood for five days and grinded in a blender. Ten g of powder was dissolved in 100 mL of methanol and incubated at 37°C for 5 days in shaker. After five days, the suspension was filtered and evaporated in fume hood. Extract, after evaporation of ethanol, was dissolved in 10% of DMSO and stored at 4°C (Mishra *et al*., 2013). Nisin (3.3g) was dissolved in 0.02N Hydrochloric acid.

Preparation of phosphatidyl choline liposome

Phosphatidyl choline (125 mg) was dissolved in 25mL of chloroform solution and 10µL of *Cuminum cyminum* and *Garcinia cambogia* extracts were added. Kept for evaporation under laminar hood. After evaporation, 100mM of Tris- HCl was added by continuous shaking. It was ultrasonicated for 4 cycles (1cycle = 10 seconds) and the solution was filtered using syringe filter and stored at 4°C (Marin *et al*., 2018).

For Nisin liposome, 76 mg of phosphatidyl choline was added in 10mL of ethanol and mixed well. 500µL of prepared Nisin was added. It was stirred in a magnetic stirrer at 50°C until the ethanol evaporated completely. Five mL of phosphate buffer saline was added (pH – 7.4) and ultrasonicated for 5 cycles and kept in ice bath for 2 minutes. Using membrane filter (0.22µm), the solution was filter sterilized and stored at 4°C for future use (da Silva Malheiros *et al*., 2012).

ANTIOXIDANT METHODS

Radical scavenging assay using DPPH

2, 2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the free radical scavenging activities of the samples. From stock solutions of samples (C, Ca, N, LC, LCa, LN), serial dilutions at concentrations from 6.25µg/mL to 50µg/mL was prepared. Each dilution of samples was added and incubated at room temperature for 30 minutes and the final volume was made up to 5 mL. The optical density was read at 517nm. The mixture without any samples was considered as control. Butylated hydroxytoluene (BHT) was taken as standard (Shahriar *et al*., 2012). The percentage of inhibition was recorded by the given formula:

$$\% \text{ of inhibition} = (1 - A_1/A_0) \times 100$$

Where, A_1 is the absorbance of the standard and A_0 is the absorbance of the control.

Scavenging activity by ABTS

2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was used to determine the scavenging activity. The freshly prepared ABTS solution (7mM) was mixed with 2.45mM of potassium persulfate. And kept in dark room for 16h. After incubation, 0.5mL of ABTS was mixed with methanol and optical density was recorded. When the absorbance reaches at 0.700 nm, then 0.5mL of solution (ABTS) was added with serially diluted samples at different concentrations ranging from 6.25 to 50µg/mL. Incubated at room temperature for 30 minutes. The absorbance was measured at 734 nm after incubation. The ABTS solution without adding samples were taken as control and BHT was standard. The percentage of inhibition was calculated by following equation (Rajurkar *et al*., 2011):

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of the blank} - \text{Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

Hydrogen peroxide scavenging activity

40mM of hydrogen peroxide was mixed with 100 mL of phosphate buffer saline (pH-7.4). 0.25mL of serially diluted samples was added at different concentrations like 6.25µg/mL-50µg/mL. One mL of hydrogen peroxide

solution was added. Incubated for 10 minutes at room temperature. After 10 minutes, the absorbance was measured at 230nm. BHT used as standard and control was prepared without adding any samples. Finally, the percentage of absorbance was measured by following formula (Shahriar *et al*., 2012):

$$\% \text{ of Inhibition} = (1\text{-}A_1/A_0) \times 100$$

Where, A1 is the absorbance of standard or extracts, and A0 is the absorbance of control.

OH radical scavenging activity

Hydroxyl radical assay was used to determine the scavenging capacity of samples (N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome cambodge). 25µL of serially diluted samples were added to 100µL of reaction mixture (2.8mM deoxyribose, 0.1mM EDTA, 1mM H_2O_2, 0.01mM ascorbate and 10mM phosphate buffer (pH-7.4)) and the final volume was made up to 1 mL by adding double distilled water. After one hour of incubation, 500µL of the mixture was taken out and 70% ethanol and 1% thiobarbituric acid was added. Placed in a water bath at 55°C for 25 minutes. Absorbance was measured at 532nm. Percentage of inhibition was determined by Awah *et al*. (2010):

$$\% \text{ of Inhibition} = (1\text{-}\ A_{0}\text{-}A_s / A_0) \times 100$$

Where, A_0 is the absorbance of the control, A_S is the absorbance of the standard or samples.

NO Scavenging activity

Sodium Nitroprusside was used to determine the nitric oxide scavenging capacity. The serially diluted samples (6.25µg/mL-50µg/mL) were added to different test tubes and to this, the mixture of sodium nitroprusside and phosphate buffer saline was added (pH-7.4). Incubated at 25°C for 150 minutes. After incubation, 0.25mL of reaction mixture was taken out and 500µL of sulfanilic acid solution was added and incubated again at room temperature for 7 minutes. Seven minutes later 0.5mL of naphthylethylenediamine dihydrochloride was added at kept in room temperature for 30 minutes. Optical density was recorded at 546 nm. Reaction mixture without adding sample considered as blank/control (Shahriar *et al*., 2012).

Scavenging capacity was assessed by following formula:

$$\% \text{ of inhibition} = (1\text{-}\ A_1/A_0) \times 100$$

Estimation of reducing power capacity

To assess the reducing power capacity, potassium ferricyanide method was followed (Yildirim *et al*., 2001). Serially diluted samples were mixed with 1.5 mL of potassium ferricyanide and 1.5 mL of 0.2M phosphate buffer saline (pH-6.6). The mixture was incubated at 50°C in a boiling water bath. After cooling down the solution, 2mL of trichloroacetic acid was added and centrifuged for half an hour at 3000 rpm. 1.5mL of suspension was collected and equal volume of double distilled water and ferric chloride solution was added. Ascorbic acid and BHT were taken as standard and PBS were used as control.

Statistical analysis

The results were presented as mean ± SD. Analysis of variance was performed and significant differences were considered at $p < 0.05$.

RESULTS AND DISCUSSION

DPPH (2, 2-diphenyl-1-picrylhydrazyl) offers a mechanism to screen the antioxidant capacity because of their radical scavenging ability mainly in plant extracts. DPPH react with certain compounds, and can able to donate a hydrogen atom. The colour of the solution is violet. When it reacts with hydrogen atom, the colour will disappear and the absorbance value will decrease. The six samples such as N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome cambodge) were tested for DPPH activity. Butylated hydroxytoluene (BHT) showed 98.6% of inhibition at concentration 50µg/mL. *Cuminum cyminum*, *Garcinia* extracts and Nisin exhibited 92 and 93 and 95 percentage of inhibition at highest concentration 50µg/mL respectively (Table 1). And the phosphatidyl choline encapsulated liposome showed higher percentage of inhibition than pure forms of samples (C, Ca, and N). This assay indicates, as the concentration increases, the percentage of absorption also increase. Nisin, Liposome Nisin, Cumin and Cambodge were significant at $p < 0.05$.

DPPH is a free radical, which has the ability to decolorize when it comes to contact with the antioxidants (Shahriar *et al*., 2012). This radical scavenging activity has been widely used technique to evaluate the antioxidant capacity especially in plants, fruits and vegetables etc. Some studies proved that, antioxidants can protect against certain diseases and have the ability to slow down the aging process (Ames *et al*., 1993). The phosphatidyl choline

encapsulated liposomes can improve the stability by testing antioxidant capacity and also safe for food preservation (Gortzi *et al*., 2007)

Table 1. Percent radical scavenging activity on the basis of DPPH for BHT (Butylated hydroxytoluene), N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome cambodge) at concentration ranging from 6.25µg/mL to 50µg/mL.

Different samples	Concentration in µg/mL				
		6.25	12.5	25	50
BHT	Mean ± SD,	98.7 ± 0.11	98.6 ± 0.11	98.56 ± 0.11	98.4 ± 0.41
N	Mean ± SD	93 ± 0	93.3 ± 0.10	93.7 ± 0.17	95 ± 0.011
LN	Mean ± SD	92.3 ± 0.49	93.4 ± 0.15	93.9 ± 0.20	94.6 ± 0.57
C	Mean ± SD	90.03 ± 1.23	91.5 ± 0.51	91.6 ± 0.52	92.03 ± 0.15
LC	Mean ± SD	92.83 ± 0.73	93.4 ± 0.36	93.5 ± 0.43	94.1 ± 0.10
Ca	Mean ± SD	90.1 ± 1.25	91.03 ± 0.92	92.7 ± 0.66	93 ± 0.86
LCa	Mean ± SD	92.6 ± 0.55	93.1 ± 0.32	93.06 ± 0.05	92.9 ± 0.85

Data reported as Mean values ± Standard Deviation (SD).

ABTS (2, 2'-Azino-bis (ethylenebenzothiazoline-6-sulfonic acid) was used to determine the scavenging capacity of extracts (**B**). In this assay, the result showed percentage of inhibition in a concentration dependent manner. The standard or BHT resulted almost 98% of antioxidant activity at highest concentration of 50µg/mL (Table 2). In ABTS assay, the samples such as, BHT, N (Nisin), LN (Liposome Nisin), C (Cumin) and LC (Liposme Cumin) were significant at $p < 0.05$.

All the samples except Ca (*Garcinia*), showed 90 % or above antioxidant activity at higher concentration. Cambodge has 81% of scavenging activity. Liposome encapsulated Garcinia resulted 90% of radical scavenging activity at 50µg/mL. Plants and fruits contained various forms of phytochemicals. Naturally produced antioxidants are much functionally stable than synthetic antioxidants (Wang *et al*., 2009). ABTS is a decolourization assay, applicable to aqueous and lyophilic systems. When the stock solution of ABTS reacts with potassium persulfate, it allows the production of ABTS radical cation. The optical density at 734nm will show the minimal absorbance due to antioxidant activity (Re *et al*., 1999).

Table 2. Percentage of scavenging activity On the basis of ABTS (2, 2'-Azino-bis ethylenebenzothiazoline-6-sulfonic acid for BHT (Butylated hydroxytoluene), N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge) at concentrations ranging from 6.25µg/mL to 50µg/mL.

Different samples	Concentration in µg/mL				
		6.25	12.5	25	50
BHT	Mean ± SD	98.0 ± 0	98.5 ± 0.11	98.63 ± 0.11	98.63 ± 0.11
N	Mean ± SD	91.63 ± 0.55	92.6 ± 0.52	93.2 ± 0.3055	94.3 ± 0.3
LN	Mean ± SD	92.6 ± 0.15	93 ± 0.1	93.6 ± 0.15	94.66 ± 0.30
C	Mean ± SD	85.4 ± 1.03	89.66 ± 1.52	91.33 ± 1.15	92 ± 1.00
LC	Mean ± SD	85.2 ± 0.25	87.6 ± 0.52	89.43 ± 0.45	90.06 ± 0.20
Ca	Mean ± SD	60.6 ± 17.89	68.46 ± 7.85	69.66 ± 10.88	81.83 ± 1.76
LCa	Mean ± SD	86.9 ± 0.31	88.0 ± 0.20	90.1 ± 0.28	90.73 ± 0.64

Data reported as Mean values ± Standard Deviation (SD).

In Hydrogen peroxide assay, the standard BHT showed 98% of antioxidant activity at highest concentration. LCa (Liposome Cambodge) and LN (Liposome Nisin) showed comparatively higher percentage of inhibition of

radical scavenging activity than other samples without liposomes. Liposome cumin and cumin resulted in the same percentage of radical scavenging activity. The percentage of scavenging capacity increased with increasing concentration of samples from 6.25µg/mL-50µg/mL (Table 3). All the samples in Hydrogen peroxide scavenging method were observed as significant at $p < 0.05$.

Hydrogen peroxide is not very much active but very toxic to cells. So, the elimination or trapping of Hydrogen peroxide is much superior aspect for the protection of food systems (Halliwell *et al*., 1987). The extracts under study have the ability to scavenge the activity of hydrogen peroxide in concentration dependent manner.

Table 3. Represent the percentage of antioxidant activity on the basis of Hydrogen peroxide assay at different concentrations from 6.25µg/mL to 50µg/mL in different samples such as BHT (Butylated hydroxytoluene, N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge).

Different samples	Concentration in µg/mL				
		6.25	12.5	25	50
BHT	Mean ± SD	98 ± 0	98.56 ± 0.11	98.56 ± 0.01	98.56 ± 0.01
N	Mean ± SD	90.8 ± 0.76	91.43 ± 0.37	92.3 ± 0.36	92.66 ± 0.15
LN	Mean ± SD	91.63 ± 0.15	92.93 ± 0.40	92.56 ± 0.32	94.43 ± 1.05
C	Mean ± SD	83.56 ± 0.51	87.3 ± 0.36	86.8 ± 4.15	93.93 ± 4.38
LC	Mean ± SD	83.73 ± 0.66	87.6 ± 1.44	90.66 ± 0.57	91.66 ± 0.57
Ca	Mean ± SD	85.3 ± 2.02	87.5 ± 0.86	88.9 ± 0.81	91.06 ± 0.28
LCa	Mean ± SD	86.6 ± 1.00	88.46 ± 0.416	90.5 ± 0.10	91.3 ± 0.10

Data reported as Mean values ± Standard Deviation (SD).

Table 4. Percentage of radical scavenging activity based on nitric acid assay at different concentrations from 6.25µg/mL to 50µg/mL in different samples such as BHT (Butylated hydroxytoluene, N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge).

Different samples	**Concentration in µg/mL**				
		6.25	12.5	25	50
BHT	Mean ± SD	98.3 ± 0.55	98 ± 0	98.33 ± 0.55	99 ± 0
N	Mean ± SD	92.6 ± 1.52	94.1 ± 0.20	94.63 ± 0.32	94.8 ± 0.15
LN	Mean ± SD	86.4 ± 0.90	91.83 ± 0.47	93.3 ± 0.30	94 ± 0.66
C	Mean ± SD	95.3 ± 0.57	94 ± 0	93 ± 0	94 ± 3.46
LC	Mean ± SD	91.8 ± 0.80	93.16 ± 0.158	93.86 ± 0.64	94.7 ± 0.55
Ca	Mean ± SD	90.3 ± 0.57	91.6 ± 1.15	92.2 ± 1.41	93.5 ± 0.32
LCa	Mean ± SD	91.6 ± 0.57	92.63 ± 0.558	93.6 ± 0.52	94.46 ± 0.41

Data reported as Mean values ± Standard Deviation (SD).

The nitric oxide scavenging activity using plant extracts showed increasing level of antioxidant activity in concentration or dosage dependent manner. Methanolic extracts of *C. cyminum* and *G. cambogia*, Nisin and encapsulated liposome samples also showed almost 94 percentage of scavenging activity at higher concentration 50µg/mL. The standard sample BHT resulted 98% of radical scavenging capacity (Table 4).

Nitric oxide is an important cell signalling molecule with a short half life in the blood stream (Lalhminghlui *et al*., 2018). When the nitric oxide radical is reacted with oxygen or superoxide, it converted into toxic form. The toxicity was reduced by the detection of scavenging activity of certain plant extracts (Shantabia *et al*., 2014). The samples except Cumin (C) were observed as significant at $p < 0.05$.

The dose of extracts depends on the scavenging activity of hydroxyl radicals. Maximum radical scavenging activity or antioxidant activity was observed at 50µg/mL concentration in each samples (Table 5). Encapsulated phosphatidyl choline liposomes indicated increased level of percentage of inhibition. In hydroxyl radical scavenging activity, N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge) were considered as significant at $p < 0.05$.

Table 5. Percentage of scavenging capacity based on hydroxyl radicals assay for different concentrations from 6.25µg/mL to 50µg/mL in various samples such as BHT(Butylated hydroxytoluene, N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge).

Different samples	Concentration in µg/mL				
		6.25	12.5	25	50
BHT	Mean ± SD	97 ±0	98 ± 1.00	98 ± 1.00	98 ± 1.00
N	Mean ± SD	92.2 ± 0.43	93.3 ± 0.51	94.2 ± 0.50	94.26 ± 0.25
LN	Mean ± SD	85.9 ± 1.05	89.6 ± 0.34	91.56 ± 0.28	92.66 ± 0.68
C	Mean ± SD	69.93 ± 0.83	78.17 ± 4.54	90.5 ± 0.17	92.4 ± 0.10
LC	Mean ± SD	83.66 ± 0.40	83.66 ± 0.40	91.32 ± 0.50	93.06 ± 0.15
Ca	Mean ± SD	46.3 ± 3.21	65.53 ± 0.92	71.4 ± 1.21	72.3 ± 1.09
LCa	Mean ± SD	90 ± 1.00	91.3 ± 0.20	92.26 ± 0.05	92.5 ± 0.10

Data reported as Mean values ± Standard Deviation (SD).

The increased activity of hydroxyl radicals cause damage to cells, which leads to physiological changes. The removal or trapping of such hydroxyl radicals prevent such damage by using certain plant extracts. The encapsulation of liposome also enhances the scavenging activity. Also some studies showed, the presence of flavanoids and phenols can increase the antioxidant activities (Lalhminghlui *et al*., 2018).

Table 6. Percentage of scavenging capacity based on reducing power capacity assay at different concentrations from 6.25µg/mL to 50µg/mL in different samples such as BHT (Butylated hydroxytoluene, N (Nisin), LN (Liposome Nisin), C (Cumin), LC (Liposome Cumin), Ca (Cambodge), LCa (Liposome Cambodge), Ascorbic acid.

Different samples	Concentration in µg/mL				
		6.25	12.5	25	50
BHT	Mean ± SD	1.137 ± 0.02	1.197 ± 0.01	1.214 ± 0.00	1.216 ± 0.00
N	Mean ± SD	0.219 ± 0.002	0.223 ± 0.007	0.240 ± 0.003	0.255 ± 0.001
LN	Mean ± SD	0.186 ± 0.007	0.2 ± 0.001	0.211 ± 0.001	0.226 ± 0.007
C	Mean ± SD	0.255 ± 0.062	0.242 ± 0.002	0.256 ± 0.007	0.276 ± 0.007
LC	Mean ± SD	0.193 ± 0.008	0.209 ± 0.004	0.228 ± 0.003	0.248 ± 0.004
Ca	Mean ± SD	0.22 ± 0.001	0.242 ± 0.002	0.256 ± 0.007	0.276 ± 0.007
LCa	Mean ± SD	0.216 ± 0.003	0.234 ± 0.004	0.267 ± 0.007	0.294 ± 0.004
Ascorbic acid	Mean ± SD	1.137 ± 0.026	1.197 ± 0.013	1.214 ± 0.004	1.216 ± 0.003

Data reported as Mean values ± Standard Deviation (SD).

In reducing power capacity assay, BHT and ascorbic acid were used as standard, and also showed better antioxidant activities other than samples. The higher the absorption value indicate more reducing power activity. All the samples exhibited reducing power activity in dose dependent manner (Table 6). All the samples other than Cumin were significant at $p < 0.05$.

The main goal of our study was to evaluate the antioxidant activity of plant extracts with nisin and also to study the activity of encapsulated phosphatidyl choline liposomes. Takahashi *et al*. (2009) published a work regarding the antioxidant properties and bioavailability of liposomes encapsulated curcumin. They stated that, liposome loaded with curcumin function as a strong and new nutrient delivery system, and also showed increased level of gastrointestinal absorption. For this research, they administered the curcumin sample in Sprague-Dawley rats. By using curcumin, the bioavailability and productivity also increased, also nanoparticles prepared from phospholipids were efficiently delivered drug into the blood stream.

Herbal plants and spices considered as better antioxidants. They are certain substance that can reduce damage due to oxidation process caused by free radicals. The methanolic extract of *Lantana camara* L. especially their

leaves, roots, and flower showed good antioxidant activity due to the presence of polyphenols. Leaf extract resulted higher antioxidant properties (Mahdi-Pour *et al*., 2012).

Our nature is blessed with wide range of herbal plants, fruits and vegetables, those are rich in antioxidants. By consuming naturally occuring or developed food preservatives, we can boost up our immunity and prevent life threatening diseases like cancer, diabetes, and blood pressure etc. In the present study, we tried to convey our ideas about antioxidant activities of certain medicinal plants *Cuminum cyminum*, *Garcinia cambogia* and a food preservative called Nisin. Also initiated an effort to evaluate the encapsulation method using phophatidyl choline liposomes.We can concluded that, the encapsulation of phophatidyl choline liposome showed increased level of antioxidant activities. And the further work is mandatory to consolidate whether the liposome encapsulated plant extracts and Nisin is applicable in food or pharma industry.

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