doi.org/10.36721/PJPS.2020.33.4.REG.2121-2129.1

Synthesis and biological evaluation of *Ellettaria cardamomum* (Cardamom) Phytosomes

Zahida Bibi[1], Nazish Jahan[1], Khalil-ur-Rehman[2] and Zill-e-Huma[1]
[1]Department of Chemistry, University of Agriculture, Faisalabad, Pakistan
[2]Department of Biohemistry, University of Agriculture, Faisalabad, Pakistan

Abstract: *Ellettaria cardamomum* (Cardamom) is an excellent antioxidant and its phytochemicals possess astounding detox properties. The bioactives of *E. cardamomum* have low bioavailability due to complex molecular structure. The main aim of this research was to formulate the *E. cardamomum* loaded phytosomes to improve the bioavailability with better ACE inhibition potential. The phytosomes were prepared by thin layer hydration technique. Various synthetic parameters for formulation of phytosomes were optimized by response surface methodology. Formulated phytosomes were characterized through spectroscopically and their biological activities eg. ACE inhibition were also investigated. Resultantly, the optimal production of phytosomes was achieved with the equal quantity (300 mg) of *E. cardamomum* and phospholipids at the time of 20 minutes of hydration. UV-Visible spectra confirmed the chemical and physical interaction between phospholipid and bioactives functional groups. The optimized *E. cardamomum* phytosome had 71% of entrapment efficiency. The average vesicle size of phytosomes was 577.8 nm with polydispersity index 0.443. SEM analysis revealed that phytosomes were spherical in shape. The phytosomes of *E. cardamomum* showed higher antioxidant and antimicrobial activities than its crude extract. The ACE inhibition activity of phytosomes (46%) was enhanced than the crude extract (39 %).

Keywords: *Elettaria cardamomum,* bioavailability, response surface methodology.

INTRODUCTION

Medicinal plants have conventionally played a pivotal role in the treatment and management of human diseases and ailments. The remedial use of medicinal plants is as old as human civilization. Because of less or no side effects, the interest in herbal products against a variety of diseases has increased all over the world (Ho *et al*., 2018). The medicinal properties of herbal drugs are credited to bioactive secondary metabolites. The herbs and its products have been used in diversified fields including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics and other industrial purposes (Singh *et al*., 2017). However, poor bioavailability and solubility of phytochemicals is problem of concern for the scientists and researchers (Lima *et al*., 2015). The poor bioavailability of phytomedicines is due to the self-aggregation, high molecular size and digestion by gastric juices (Yang *et al*., 2015). Multiple options like polymeric nanoparticles, liposomes, phytosomes, nano-shells, nano-emulsions, transferosomes and various other medicinal formulations are possible for overcome the bioavailability problems (Lewandowska *et al*., 2016). The phytosome formation is the most effective method for enhancing bioavailability. Phytosomes are basically phyto-phospholipid complexes which are of polyphenols and phospholipids (Vu *et al*., 2018).

**Corresponding author:* e-mail: zilehuma098@gmail.com

All around the world, hypertension is the strongest cardiovascular risk factor and increasingly serious public health menace. A Zinc metal protease is the Angiotensin-I-converting enzyme (ACE) that plays an effective role for maintaining blood pressure normal by catalyzing the transformation of angiotensin I to a vasoconstrictor angiotensin II and also enhancing the degradation of vasodilator (bradykinin) (Paiva *et al*., 2016). ACE is an effective drug target for management of hypertension. Hypertention is mostly treated with synthetic ACE inhibitors but remains poorly controlled and showed large side effects. Now a days, the green ACE inhibitors as alternative of synthetic hypotensive drugs being equally potent, but with less or no less side effects are being introduced.

Cardamom (*E. cardamomum*) commonly known as queen of spices belongs to the family of *Zingiberaceae*. *E. cardamomum* being an amazing antioxidant contains medicinally important of flavonoids, terpernoids, alkaloids, aromatic compounds, sterols, lipids and several minerals (Mojgan and Roya, 2016). It is used for treatment of digestive, cardiac, kidney and pulmonary diseases due to their antioxidant, antimicrobial and anticancer potential (Abu-tawil, 2017). The aim of this study was the synthesis and characterization of *E. cardamomum* phytosome to enhance the bioavailability and ultimately to improve its antimicrobial, antioxidant and ACE inhibition potential. Different synthesis parameters were optimized by response surface methodology (RSM) to formulate optimized phytosomes with maximum wavelength.

Methodology

Preparation of extract

E. cardamomum fruits were purchased from authentic herbal store of Faisalabad and identified by plant taxonomist. Powder of *E. cardamomum* (25g) were placed in a cellulose extraction thimble and 300 ml of n-hexane was used as defatting solvent. The defatted powder was treated with 300ml of methanol for extraction through Soxhlet Apparatus. Extract was filtered through filter paper and concentrated through rotary evaporator (Jahan *et al*., 2016).

Formulation of E. cardamomum phytosomes

The *E. cardamomum* phytosome was prepared by thin layer hydration technique consists of two steps: Firstly, *E. cardamomum*– soy phosphatidylcholine (SPC) complexes were prepared by dissolving various amounts (100-500 mg) of *E. cardamomum* and soy phosphatidylcholine in ethanol (20 ml). The mixture was stirred at a temperature 25^{o}C for 120 minutes. The ethanol was evaporated and dried residues of *E. cardamomum* SPC complexes (0.2%) were redissolved in anhydrous ethanol, rotated in flask of rotary evaporator at 180 rpm, 40^{o}C for 30 minutes. The casted film was dissolve in phosphate buffer salin (PBS, pH 7.4) and kept for 15-20 minutes for hydration. After then lipid complexes swelled was peeled off from the wall of flask. At the last the formulated phytosomes suspension was sonicated for 4 minutes (Maryana *et al*., 2016; Freag *et al*., 2018).

Optimization of E. cardamomum phytosomes formulations

Response surface methodology (RSM) was used to optimize the effect of parameters on synthesis of *E. cardamomum* phytosome. Central composite design of RSM was used to generated the design of 20 experiments for optimization of parameters. The independent parameters selected for optimization were the concentration of *E. cardamomum* extract (100-500 mg), concentration of soy phosphatidylcholine (100-500 mg), hydration time (15-20 mins.) and the dependent variable was maximum wavelength (λ_{max}). The responses obtained after the preparation of formulations was analyzed by Design expert, version 6.0.8 portable.

Characterization of E. cardamomum phytosomes

The vesicle size and polydispersity index *E. cardamomum* phytosomes were examined by Dynamic light scattering spectroscopy. The sample was taken into the cell and the size of vesicle and size of vesicle distribution were measured by the zeta sizer at 0-20% of light intensity (Maryana *et al*., 2016). The evaluation of surface morphology of *E. cardamomum* phytosomes was done by Scanning Electron Microscope (JSM6100).

Entrapment efficiency of formulated phytosomes

For determination of entrapment efficiency of optimized *E. cardamomum* phytosomes, 1.5mL of formulated samples was centrifuged for 60 minutes at room temperature, and supernatant was collected by using micropipette. It was dissolved in methanol and different dilutions (20-100µg/ml) were made. The absorbance of all samples was measured by UV-spectrophotometer at 302 nm in order to determine the amount of free and entrapped drug contents in phytosomes. Entrapment efficiency was calculated by the following formula (Marayana *et al*., 2016).

$$\text{Entrapment efficiency (\%)} = \frac{\text{amount of encapsulated } E.\,cardamomum}{\text{amount of total } E.\,cardamomum} \times 100$$

Antioxidant activity of E. cardamomum phytosomes

Antioxidant activity of *E. cardamomum* phytosomes was evaluated as following different methods.

2,2-Diphenyl-picrylhydrazyl (DPPH) assay

Five different concentrations (20-100µg/mL) of *E. cardamomum* extract, its phytosomes and ascorbic acid were prepared. Freshly prepared DPPH solution in methanol (1mL, 0.1mM) was added and kept for 30 minutes in dark. The absorbance was noted at 517nm. The ascorbic acid was used as standard. The following formula was applied to determine the inhibition of DPPH radical (Moe *et al*., 2018).

DPPH inhibition (%) = $[1\text{-}A_I/A^0] \times 100$

Where; A_I= Absorbance of sample: A^0 =Absorbance of control

Linoleic acid assay

For this assay, a reaction mixture consisting samples (500 µg), buffer (2 ml, 0.04 M, pH 7), linoleic acid emulsion (2.5mL) was prepared and kept at 37^{o}C for 72 hours. After each 24 hours, 2mL of the incubated sample (*E. cardamomum* extract, phytosomes and ascorbic acid) was taken and 0.5mL of 30% (w/v) ammonium thiocynate and 0.5mL of $FeCl_2$ (0.02 M) was added. Amount of peroxide was assessed by measuring the absorbance at 500 nm. The ascorbic acid was utilized as standard (Jahan *et al*., 2016).

% Inhibition of lipid peroxidation = $100\text{-}[A_I/A^0 \times 100]$

Reducing power assay

Different concentrations (20-100µg/ml water) of *E. cardamomum* extract, phytosome and ascorbic acid were prepared. Equal volumes (2.5ml) of potassium ferricyanide (1%) and phosphate buffer (2.5ml, 0.2M, pH 6.6) was mixed in each sample (2.5ml) in test tube. The control contained all the reaction reagents except the sample. In oven the mixture was kept for 20 minutes at 50 ^{0}C. The reaction was stopped by addition of TCA (2.5ml 1 % w/v) and centrifuged for 10 minutes at 3000 rpm. The upper layer (2.5ml) of centrifuged mixture was diluted with water. Absorbance of this solution was noted at 700 nm after the addition of 0.5ml ferric chloride solution (0.1 % w/v).

Table 1: Response of experiments designed by CCD

S. no	*E. cardamomum* conc. (mg)	SPC conc.(mg)	Hydration time (mins.)	λ_{max} (nm)
1	100	100	15	212
2	-36.36	300	17.50	309
3	500	500	20	318
4	100	100	15	245
5	300	300	20	324
6	300	636.36	17.50	267
7	100	100	15.00	212
8	500	500	15.00	312
9	300	300	17.50	287
10	300	300	13.30	289
11	500	100	15.00	256
12	300	300	17.50	287
13	300	300	21.70	235
14	636.36	300	17.50	290
15	100	500	15.00	256
16	300	300	17.50	287
17	500	300	20	305
18	300	300	17.50	287
19	300	300	17.50	287
20	300	-36.36	17.50	302

CCD= central composite design: SPC = soy phosphatidylcholine

Table 2: Analysis of variance (ANOVA)

Source	Sum of squares	Df	Mean square	F Value	Probe>F	
Model	28614.62	9	3179.40	16.84	<0.0001	Significant
A	16940.78	1	16940.78	89.73	<0.0001	
B	92.75	1	92.75	0.49	0.4993	
C	1181.00	1	1181.00	6.26	0. 0314	
AB	5460.12	1	5460.12	28.92	0.0003	
AC	703.12	1	703.12	3.72	0.0825	
BC	435.12	1	435.12	2.30	0.1599	
A^2	3292.75	1	3292.75	17.44	0.0019	
B^2	228.14	1	228.14	1.21	0.2974	
C^2	189.13	1	189.13	1.00	0.3405	
Residual	1887.93	10	188.79			
Lack of Fit	677.09	5	135.42	0.56	0.7305	Not significant
Pure error	1210.83	5	242.17			
Cor total	30502.55	19				

Table 3: Antimicrobial activity of *E. cardamomum* phytosome

	Zone of inhibition (mm)		
	Conc. (mg)	*B. subtilis*	*E.coli*
E. cardamomum Phytosome	5	6	5
	100	10	8
	150	13	14
E. cardamomum extract	50	4	3
	100	7	7
	150	11	10
Ciprofloxacin (positive control)	50	17	9.5
	100	18	7
	150	20	10

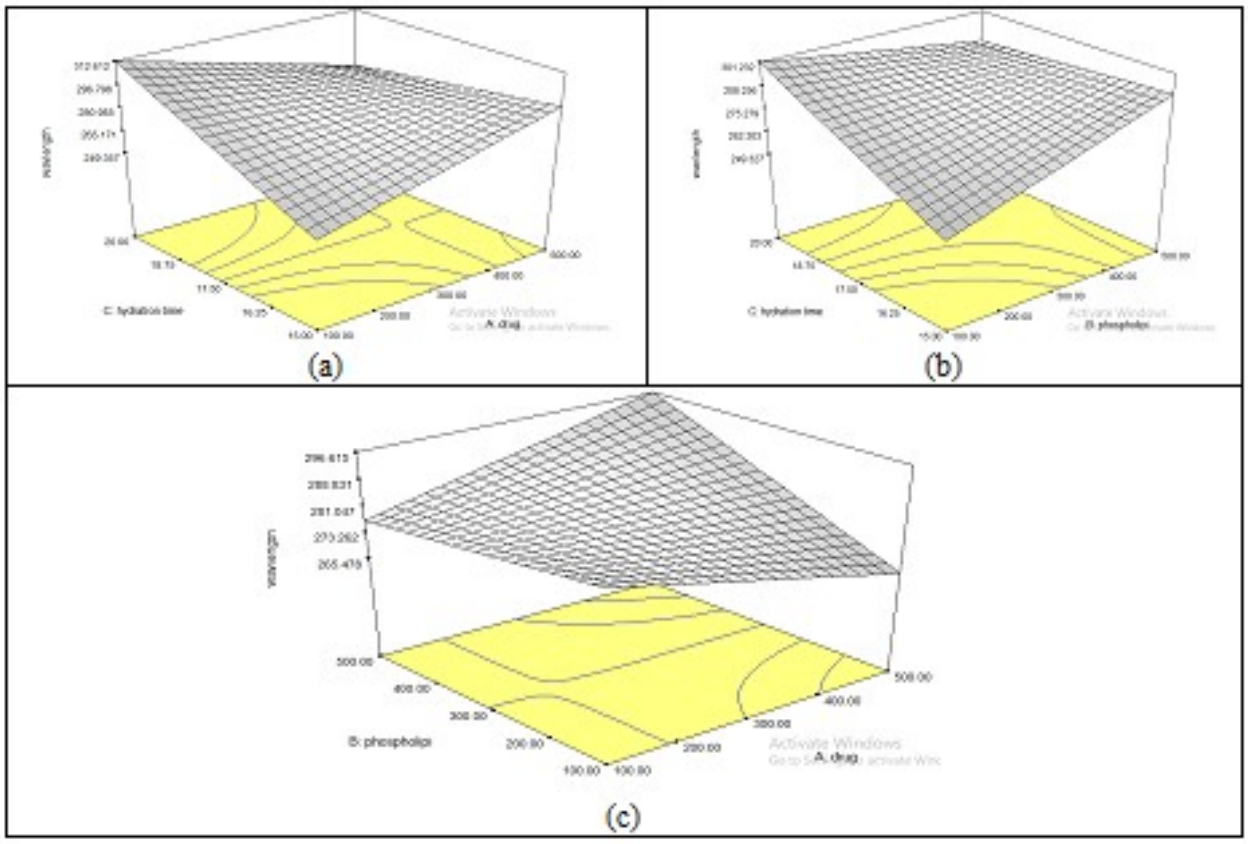

Fig. 1: (a), (b) and (c) response surface graphs for the consequence of *E. cardamomum* conc., SPC conc. and hydration time on λ_{max}

Nitric oxide scavenging assay

The solution of sodium nitro-pruside (1mL, 5mM) prepared in buffer (0.2 M, pH 7) was mixed with different concentrations (20-100µg/ml) of plant extract and phytosome of *E. cardamomum* and kept for 30 minutes. After completion of time, 1.5ml of incubated solution was separated and 1.5ml of Griess reagent was added (1% sulphonamide, 2% phosphoric acid, 0.1% coupling reagent). The absorbance was noted at 50nm. Analysis of ascorbic acid was taken as standard. Inhibition percentage of radical assay was calculated with the following formula (Jahan *et al*., 2016).

$$\%\text{inhibition of radical} = [A^0\text{-}A_1/A^0] \times 100$$

Superoxide radical scavenging assay

Five different concentration (20-100µg/ml) of *E. cardamomum* extract, phytosome and ascorbic acid was prepared. Plant extract, phytosome and standard (1mL) was mixed with 1mL of sodium carbonate, (5%), 0.4 ml of NBT (150µL) and 0.3 ml of EDTA (0.5%). The first absorbance was observed at 560 nm. Hydroxyl amine hydrochloride (0.4ml, 1%) was added for beginning reaction again in the above solution. The mixture was kept for 5 minutes at 25 ^{0}C. NBT reduction was measured at 560 nm. Ascorbic acid was used as standard. Inhibition percentage of radical assays was calculated with the following formula (Moe *et al*., 2018).

$$\text{Inhibition (\%)} = [1\text{-}A_1/A^0] \times 100$$

Antimicrobial activity

The antimicrobial activity of *E. cardamomum* phytosomes and *E. cardamomum* extract was evaluated by well diffusion method against gram-positive (*Bacillus subtillus*) and gram-negative strains (*Escherchia coli*). Nutrient agar was dissolved in distilled water pH at 7 was adjusted and autoclaved for sterilization at 121^{o}C for 15 minutes. Sterilized medium (15 mL) was spreaded into the petri plates uniformly in the form of thin film of gel (2-3 mm thickness) and incubated overnight in oven at 37^{o}C. Different conc. (50,100,150mg/ml) of *E. cardamomum* phytosomes, *E. cardamomum* extract and ciprofloxacin (positive standard) were prepared by dissolving sample in 1mL of distilled water. In well (6 mm) 10µl of each solution was applied and allowed to dry solvent in antiseptic hood. Antibiotic ciprofloxacin was used as standard. Bacterial cultures were inserted on sterilized petri plates. Plates were incubated for 24 hours at 37^{o}C. At the end of incubation, the zone of inhibition growth (mm) were measured by zone reader (Al-Ansari *et al*., 2019).

ACE inhibitory activity

Rabbits were purchased from the market, the healthy lungs were removed from freshly slaughtered rabbits and washed with saline solution (0.8%). Phosphate buffer saline was utilized to grind the washed lungs and centrifuged at 4000 rpm for 10 minutes. The upper layer was separated out and residues were washed several times with acetone along with continuous stirring on a magnetic stirrer for dehydration. Excess of acetone was removed and residues were kept overnight for drying. Dried material was ground to fine powder and stored at 4°C. Lung acetone powder (0.5g) was mixed with Borate buffer (10ml, 100mM) having pH 8.3. The mixture was stirred overnight on a magnetic stirrer and centrifuged at 4000 rpm for 45 mins. Supernatant was dialyzed with borate buffer by using dialyzing membrane and lyophilized to remove excess water.

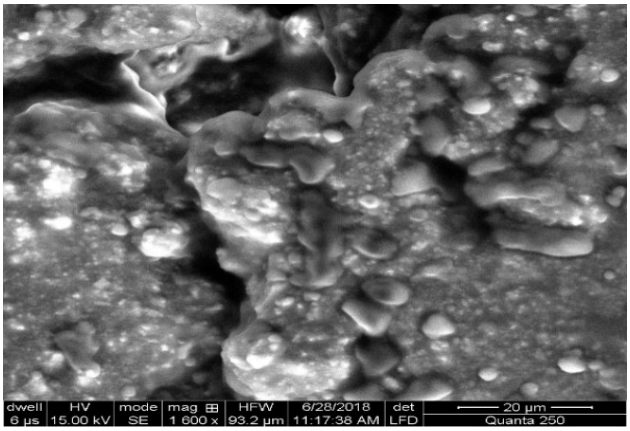

Fig. 2: Scanning electron microscopy photomicrograph of *E. cardamomum* phytosomes

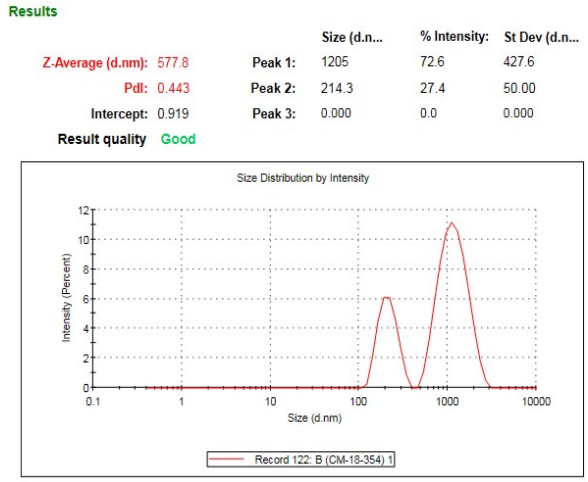

Fig. 3: Particle size of *E. cardamomum* phytosomes

The ACE solution (50µL of 100 U/ml) was mixed with 50 µL of *E. cardamomum* crude extract and phytosomes, separately incubated at 37°C for 10 minutes. A solution (150 µL) was prepared by mixing 8.3 mM Hip-His-Leu in borate buffer and HCL (250 µL of 1 M). Two types of solutions, like blank and reaction were prepared for each sample of crude extract and phytosomes. In blank solution only HCL (250µL of 1M) and in reaction solution substrate was added incubated for 80 minutes at 37°C. In reaction solution the reaction was stopped by adding 250 µL HCL (1M). The resulting hippuric acid was separated by utilizing ethyl acetate (1500µL) and centrifuged at 4000 rpm for 15 minutes. After centrifugation 750µL supernatant was separated in test tubes. The solution was dried under air flow at 7°C. Dried material was mixed with 1mL of distilled water in a test tube and used to determine absorbance at 228 nm by utilizing UV/Visible spectrophotometer. The ACE inhibition percentage of *E. cardamomum* phytosomes, its crude extract and captopril were calculated according to the formula:

%I ACE = 100[(A-B)-(C-D)]/ (A-B)

Where, A = absorbance in the presence of ACE, B = absorbance of reaction blank, C = absorbance in the case of ACE and inhibitors, D = absorbance of blank reaction.

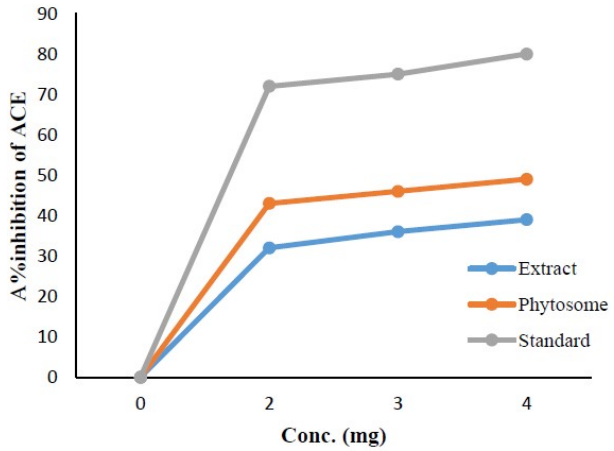

Fig. 4: %inhibition of *E. cardamomum* phytosomes against ACE

RESULTS

Optimization of formulation parameters by response surface methodology

The *E. cardamomum* phytosomes were prepared by thin layer hydration technique to enhance the bioavailability and therapeutic efficacy. The *E. cardamomum* phytosomes were formulated by using *E. cardamomum* extract, soy phosphatidylcholine and ethanol (Maryana *et al*., 2016). Various formulation The *E. cardamomum* phytosomes were scanned at wavelength 200-400 nm by UV-visible spectrophotometer and λ_{max} (nm) were determined. The *E. cardamomum* phytosomes showed higher λ_{max} (nm) at 324 nm and optimized phytosomes was used for further study. The higher value of λ_{max} (nm) of any product showed maximum drug entrapped to show high biological activities. The response of designed experiments by CCD is shown in table 1. The maximum value of λ_{max} (324 nm) of *E. cardamomum* phytosomes

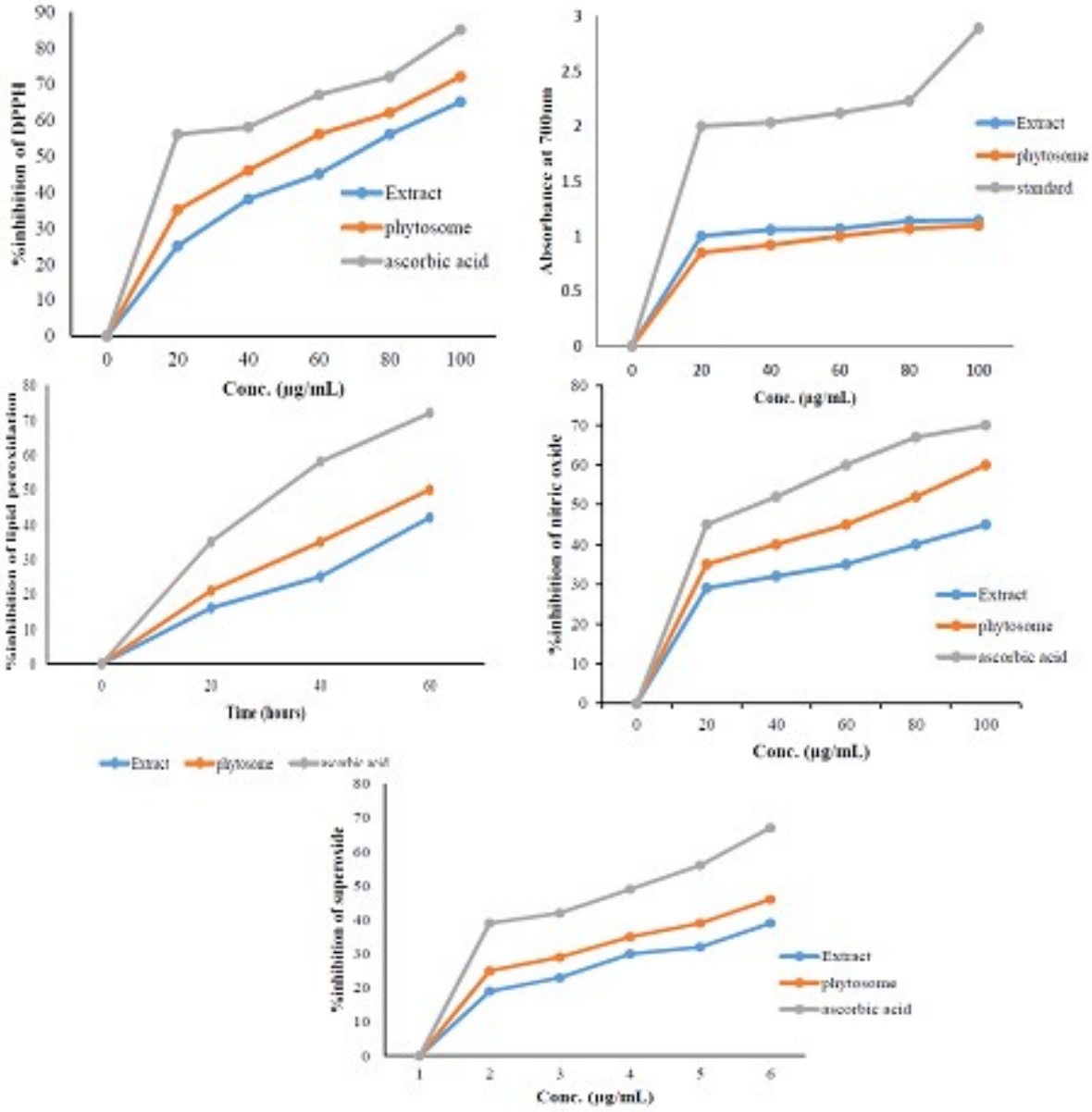

Fig. 5: (a) % Inhibition of DPPH radical (b) Reducing power (c) Lipid per oxidation inhibition (d) Nitric oxide assay (e) Super oxide assay

was observed at the concentration of 300 mg of both (*E. cardamomum* and phospholipid) and 20 minutes hydration time. The statistical experimental design suggested that quadratic model was most suitable model with the smallest p value and F value.

Analysis of variance (ANOVA) was used to identify significant terms in quadratic model. It revealed the contribution of each variable on overall estimated response (Zafar *et al*., 2018). P-value less than level of significance (p<0.05) suggested that the quadratic model was significant. The contribution of each parameter described in terms of F-ratio. For synthesis of *Ellettaria cardamomum* phytosome the F-value was 16.84 suggested quadratic model was significant. The model terms like A, B, C, A^2, B^2, C^2, AB, AC, and BC was significant if "prob > F" was less than 0.05. If "probe >F" value greater than 0.1000 indicated that the model terms were not significant. In this study, quadratic terms A^2 (p = 0.0019), A (p=0.0001), C (p=0.0314) and interaction terms AB (p = 0.0003) were statistically significant model terms (table 2).

Effect of independent variables on response variable

The response surface graph was generated using design expert (version 6.0.8 portable) on the basis of quadratic

regression equation. The response surface graphs revealed the effect of different concentrations of *E. cardamomum*, phospholipid and of hydration time on response wavelength (nm) for the synthesis of *E. cardamomum* phytosomes. With the increased conc. of *E. cardamomum* from 100 to 300mg, the λ_{max} value of phytosomes was also increased. Further increase in the concentration of *E. cardamomum*, decreased the λ_{max} value of its phytosomes. Similar trend of phospholipid concentration was observed, the λ_{max} value of phytosomes was increased from 100-300 mg concentration of phospholipid and decreased with further increase of phospholipid conc. to 500 mg. The hydration time showed positive effect on λ_{max} (nm). The λ_{max} value of *Elettaria cardamomum* phytosomes was increased with increase in the studied hydration time from 15-20 minutes. (fig. 1 a, b and c).

Entrapment efficiency

The entrapment efficiency of optimized *E. cardamomum* phytosome was determined by using ascorbic acid as standard. The standard curve of ascorbic acid was used to determine the concentration of free drug. With the increase in conc. of phospholipid the entrapment of drug was also increased. The entrapment efficiency of optimized phytosomes was upto 74%. The phytosomes with highest entrapment efficiency was formulated with the equal concentration (Ratio 1:1) of *E. cardamomum* and phospholipid.

Characterization of E. cardamomum phytosomes

SEM images of synthesized *E. cardamomum* phytosomes were found spherical in shape (fig. 2). The photomicrograph of phytosome surface indicated that there were no crystalline structures or any impurity on the surface. Most of the drugs having crystal structure when complexed with phospholipid are changed into molecularly dispersed or amorphous form.

In this study, the optimized *E. cardamomum* phytosomes formulated by 300 mg of *E. cardamomum* extract and 300 mg of SPC, showed average particle size of 577.8 nm and polydispersity index 0.443 (fig. 3). The polydispersity index value less than 0.5 of *E. cardamomum* phytosomes (0.443) indicated the homogeneity and uniformity of vesicle size in vesicle system.

ACE inhibition potential

ACE inhibition activity has made the phenolic compounds an encouraging alternative of synthetic drugs and naturally originating bioactive food components (Lacroix *et al*., 2016). The *E. cardamomum* phytosomes showed the higher (46 %) ACE inhibition activity at the 4 mg/ml concentration as compared to *E. cardamomum* extract (39%). ACE inhibition activity of captopril was 80% at the same dose of 4 mg/ml. Results revealed that captopril has more ACE inhibition activity followed by phytosomes (fig. 4).

Antioxidant potential

The *E. cardamomum* phytosomes demonstrated significantly high antioxidant potential as compared to *E. cardamomum* extract in all studied antioxidant assays. In this study different assays of antioxidant activity were performed and the finding have been graphically presented in the fig. 5.

2, 2-Diphenyl-1-picrylhydrazyl assay of *E. cardamomum* phytosomes showed higher (72%) radical quenching activity at the 100µg/ml conc. as compared to *E. cardamomum* extract (65%). The DPPH radical-scavenging activity was measured by reducing the stable, violet DPPH radical to the yellow DPPH-H. The degree of color change depends on the antioxidant potential of the studied samples through their hydrogen-donating ability. The sample with the strong antioxidant potential have the high % of radical-scavenging activity. The reducing power assay (1.145) of *E. cardamomum* phytosomes at 100µg/ml was lower than the ascorbic acid (2.98). The *E. cardamomum* extract showed lower reducing power (1.098) as compared to phytosomes and ascorbic acid. The lipid peroxidation inhibition of *E. cardamomum* phytosomes (50%) and of extract (42%) at 72 hours was lower than the ascorbic acid (72%). *E. cardamomum* phytosomes showed enhanced nitric oxide and super oxide inhibition as compared to the crude extract, but this antioxidant potential was relatively less than the standard ascorbic acid.

Antimicrobial activity

The antimicrobial activity of *E. cardamomum* phytosomes was evaluated quantitatively by well diffusion method against gram positive (*B.subtilis*) and gram negative (*E. coli*) strains. The results revealed that phytosomes showed higher zone of inhibition at 150 mg conc. (*B. subtilis* 13 mm; *E.coli* 14 mm) as compared to the crude extract (*B. subtilis*, 10mm; *E.coli* 11mm). The antimicrobial potential of the formulated phytosomes as comparable to the standard drug (ciprofloxacin) at the concentration of 150 mg against both treated strains (table 3). By comparing antimicrobial activity of *E. cardamomum* phytosomes and extract, it was evaluated that phytosomes of *E. cardamomum* showed higher antimicrobial activity. The increased antimicrobial activity of *E. cardamomum* in complexing with phospholipid may be increased polarizeability of hydroxyl groups of flavonoids (Al-Ansari *et al*., 2019).

DISCUSSION

The *E. cardamomum* phytosomes were prepared to enhance the bioavailability and therapeutic efficacy Response surface methodology (RSM) being the most popular optimization method in the recent years was the tool of choice for optimal formation of phytosomes. The purpose of optimization was to formulate a product with

good characterization and high biological activities. The UV analysis of phytosomes showed some changes in absorption as compared to *E. cardamomum* extract and phospholipid spectra. It was reasonable to assume that some interactions were developed between functional groups of *E. cardamomum* and phospholipid contents during phytosomes formation.

Entrapment efficiency of several drugs becomes better when ratio 1:1 of drug and phospholipid is used. During the interaction with the hydrophilic head of the phospholipid oriented towards the water loving area and hydrophobic oriented water hating area. As a result, off these interactions with drug phytosomal complexes were formed (Singh *et al*., 2018). The SEM image of the phytosomes was due to interactions of hydrogen bond and electrostatic interaction (Zhang *et al*., 2018). The small size enhanced the ability of *E. cardamomum* phytosome to cross cell permeable membrane to enhance the bioavailability of *E. cardamomum* (Maryana *et al*., 2016). The nature and quality of phenolic compounds have direct correlation with ACE inhibition activity (Zhang *et al*., 2018). *E. cardamomum* contained appreciable amount of flavonoids and anthocyanin, due to which this plant showed good ACE inhibition activity. In the formulated phytosomes, the bioavailability of flavonoids and anthocyanins increased, so the ACE inhibition activity was also increased (Ghanbarzadeh *et al*., 2016).

The flavonoids and polyphenols are multifunctional antioxidants. These compounds reduce the free radicals by donating a hydrogen radical (Al-Ansari *et al*., 2019). Positive correlation was always found between total phenolic contents and antioxidant potential (Jayathilake *et al.,* 2016). The polar groups of phospholipids may cause the enhanced antioxidant potential of *E. cardamomum* in phytosomes. In *E. cardamomum* the presence of vitamin C, riboflavin, thiamin and some phytochemicals are the contributory antioxidant potential (Sharabi *et al*., 2018).

CONCLUSION

The formulated *E. cardamomum* phytosomes showed better ACE inhibition, antioxidant and antimicrobial potential as compared to its crude extract. The phytosomes being novel drug delivery system therefore, may be proved as a drug of choice with better bioavailability and curative potential to combat the hypertension and other biological disorders.

REFERENCES

Abu-tawil GM (2017). Cardamom (*Elettaria cardamomum*) perinatal effects on the development, behavior and biochemical parameters in mice offspring. *Sau. J. Bio. Sci.,* **4**: 56-61.

Al-Ansari M, Al-Humaid LA, Vijayaraghavan P, Ravindran B, Chang SW, P Agastian, Rathi MA and Balamuralikrishnan B (2019). Identification of phytochemical components from *Aerva lanata* (Linn.) medicinal plants and its *in vitro* inhibitory activity against drug resistan microbial pathogens and antioxidant properties. *Saudi J. Biol. Sci*. **26**(6): 1129-1133. https://doi.org/10.1016/j.sjbs.2019.02.010.

Freag MS, Saleh WM and Abdallah OY (2018). Laminated chitosan-based composite sponges for transmucosal delivery of novel protamine-decorated tripterine phytosomes: Ex-vivo mucopenetration and in-vivo pharmacokinetic assessments. *Carbohydrate Polymers,* **188**: 108-120.

Ghanbarzadeh B, Babazadeh A and Hamishehkar H (2016). Nano-phytosome as a potential food-grade delivery system. *Food Bioscience,* **15**: 126-135. https://doi.org/10.1016/j.fbio.2016.07.006

Ho PJ, Sung JJ, Cheon KK and Tae HJ (2018). Anti-inflammatory effect of *Centella asiatica* phytosome in a mouse model of phthalic anhydride-induced atopic dermatitis. *Phytomedicine,* **1**(43): 110-119.

Jahan N, Rahman K, Basra SMA, Sajid S and Afzal I (2016). Seed Enhancement of *Silybum marianum* and Optimization of Silymarin Extraction. *Int J Agric & Biol,* **18**(2): 464-470.

Jayathilake C, Rizliya V and Liyanage R (2016). Antioxidant and free radical scavenging capacity of extensively used medicinal plants in Sri Lanka. *Procedia Food Science,* **6**: 123-126.

Lacroix IME, Meng G, Cheung IWY and Li-Chan ECY (2016). Do whey protein-derived peptides have dual dipeptidyl-peptidase IV and angiotensin I-converting enzyme inhibitory activities. *J Funct Foods,* **21**: 87-96.

Lewandowska U, Fichna J and Gorlach (2016). Enhancement of anticancer potential of polyphenols by covalent modifications. *Biochem. Pharmaco.,* **109**: 1-13.

Lima TC, Diego Santos A, Costa DTM, Souza RJ, Barisonb A, Mario Steindel and Biavatti MW (2015). Chromenes from leaves of *Calea pinnatifida* (R. Br.) Less. And evaluation of their leishmanicidalactivity. *Revi. Brasil. de Farmaco.*, **12**(1): 67-88.

Maryana W, Rachmawatib H and Mudhakir D (2016). Formulation of phytosome containing Silymarin using thin layer-hydration technique aimed for oral delivery. *Mater. Tod.*: *Proceedings,* **3**(3): 855-866.

Moe TS, Win HH, Hlaing TT, Lwin WW and Htet ZM and Mya KM (2018). Evaluation of *in vitro* antioxidant, antiglycation and antimicrobial potential of indigenous Myanmar medicinal plants. *J. Integr. Med.,* **16**(5): 358-366.

Mojgan O and Roya S (2016). Evaluation of antibacterial activity of silymarin against enteric bacterial pathogens. *Int. J. Herb. Med.,* **4**(5): 44-45.

Paiva B, Cedena M, Puig N, Arana P, Vidriales M, Cordon L, Flores-Montero J, Gutierrez NG, Martín-

Ramos M, Martinez-Lopez J, Ocio EM, Hernandez MT, Teruel A, Rosiñol L, Echeveste M, Martinez R, Gironella M, Oriol A, Cabrera C, Martin J, Bargay J, Encinas C, Gonzalez Y, Dongen JJMV, Orfao A, Blade J, Mateos M, Lahuerta JJ and Miguel JFS (2016). Minimal residual disease monitoring and immune profiling in multiple myeloma in elderly patients. *Blood,* **127**(25): 3165-3174.

Sharabi A, Tsokos MG, Ding Y, Malek TR, Klatzmann D and Tsokos GC (2018). Regulatory T cells in the treatment of disease. *Nat. Rev. Drug Disc*., **17**: 823-844.

Singh RP, Gangadharappa HV and Mruthunjaya K (2018). Phytosome complexed with chitosan for gingerol delivery in the treatment of respiratory infection: In vitro and in vivo evaluation. European. *J. Pharma. Sci.*, **122**: 214-229.

Singh RP, Gangadharappah HV and Mruthunjava K (2017). Phospholipids: Unique carriers for drug delivery systems. *J. of drug delivery Sci. and Tech.,* **39**: 166-179.

Vu HTH, Hook SM, Siqueira SD, Müllertz A, Rades T and McDowell A (2018). Are phytosomes a superior nanodelivery system for the antioxidant rutin? *Int. J. Pharm.,* **548(1)**: 82-91.

Yang G, Zhao N, Feng Y, Zhang Y, Liu Y and Dang B (2015). Improved dissolution and bioavailability of silymarin delivered by solid disoersionn prepared using supercritical fluids. *Asi. J. Pharm. Sci.,* **10(3)**: 19-202.

Zafar F, Jahan N, Rahman K and Bhatti HN (2018). Increased oral bioavailability of piperine from an optimized piper nigrum nanosuspension. *Planta medica,* DOI 10.1055/a-0759-2208.

Zhang Y, Pechan T and Chang SKC (2018). Antioxidant and angiotensin I-converting enzyme inhibitory activities of phenolic extracts and fractions derived from three phenolic-rich legume varities. *J. Func. foods,* **42**: 289-297.