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Research Article

Anti- Hypertensive Drug: Formulation And Characterization of Foeniculum Vulgare Phytosomes to Enhance the Bioavailability and ACE Inhibition Potential of Secondary Metabolites

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ABSTRACT

Fennel's dry seed has historically been proven as an anti-inflammatory, analgesic, anti-caries, antispasmodic, and diuretic to treat glaucoma, galactagogue, and high blood pressure. *F. vulgare* has hepato-protective flavonoids and organic compounds/secondary metabolites with low bioavailability. This study was done to formulate the *F. vulgare*-loaded phytosomes to improve the bioavailability and therapeutic/ACE inhibition potential of *F. vulgare*. *F. vulgare* phytosomes were designed by the thin layer hydration (TLH) technique. These phytosomes were characterized by UV-Vis and FTIR Spectroscopies. The in-vitro biological study was also conducted. The high encapsulation efficiency (95%) was obtained at optimized parameters (*F. vulgare* 1500 mg; phospholipid 1500 mg; stirring time 240 min.) and 259 nm λ_{max} . UV-visible and FT-IR spectra of phytosomes vindicated chemical and physical interactions between functional groups of *F. vulgare* and phospholipid. Phytosomes have unveiled the highest 74% ACE inhibition potential at 2.5mg/ml than crude extract and are closely related to the standard. *F. vulgare* phytosomes also showed excellent antioxidant activity as compared to *F. vulgare* ($P < 0.05$) proving phytosomes more suitable for application.

INTRODUCTION

Secondary metabolites of plants made an important contribution to the treatment of numerous diseases including cancer, infections, hypertension and inflammation, etc. In plants, secondary metabolites could be classified into the following chemically described groups: compounds of terpenes, phenolics, nitrogen,

flavonoids and sulfur, etc. (Khare et al., 2020). Since ancient times, the active ingredients from natural resources (plants) have been used to cure above mentioned diseases (Çelik & Gürü, 2015). Many active ingredients in plant extract have much less absorption properties when taken orally (Teng et al., 2012). Their poor absorption is due to two main characteristics: (1) the oversized

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structure of compounds couldn't be absorbed because of passive diffusion or inert absorption, and (2) the solubility of lipids and water through the wall membrane of the gastrointestinal tract is low (Göke et al., 2018).

The physiological stress promotes obesity, cardiovascular, neurological, and autoimmune diseases, and the aging process (Simioni et al., 2018). Throughout this process, endogenous and exogenous production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) increases (Martemucci et al., 2022) while antioxidants reduce the free radicals and neutralize these oxidants. Antioxidants as reducing agents and metal chelates help to convert hydroperoxides into stable compounds and their binding of proteins, transferrin, metallothionein, and ceruloplasmin tackle the binding of pro-oxidant metals (iron and copper). (Hussain & Kayani, 2020). Ingesting antioxidants in plants helps to prevent damage caused by ROS (Ali, Ahsan, Zia, Siddiqui, & Khan, 2020). That's why, the antioxidant activity of the plants is attributed to the secondary metabolites present in them (Lee, Lin, Yu, & Lee, 2017).

Phytosomes are also known as phyto-phospholipids which serve as a bridge between conventional delivery and novel delivery systems. Indena facilitates the assimilation of plant extract or water-soluble phyto-constituents into phospholipids to form lipid-compatible complexes to enhance the bioavailability and absorption of active ingredients (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007). Production of phytosomes initiates when phospholipids are molecularly bound to the active ingredients of a plant. The phospholipid consists of a water-soluble head and fat-soluble tail that serve as an effective emulsifier (N. Jain et al., 2010). Phytosomes are more effectively integrated into the body and give improved results than traditional herbal extracts

(Manthana & Srinivas, 2010); (AGRAWAL, GUPTA, & CHATURVEDI, 2012).

For the treatment of primary hypertension, Angiotensin-converting enzyme (ACE) inhibitors were widely prescribed (Wang et al., 2014). Considering its critical role in hypertension and cardiovascular diseases, ACE inhibitors are an attractive target for drug design (Yakimova, Petkova, & Stoineva, 2017). The renin-angiotensin system (RAS) recreated an important role in an interconnected collection of a mechanism for regulating blood salt homeostasis length and may play a role in pathogen sizing aspects of metabolic syndrome (Bawa et al., 2019). ACE is a core component of the RAS, which controls blood pressure by regulating the amount of fluid in the body (Casarini et al., 2012).

Fennel (*Foeniculum vulgare* Miller), a perennial herb of the Apiaceae family, is grown mainly in the northern hemisphere's temperate regions with culinary and medicinal uses. The main chemical ingredients of fennel are flavonoids, polyphenols, carotenoids, minerals, and vitamins (Koppula & Kumar, 2013). Fennel dry seed has historically been used as an anti-inflammatory, analgesic, antispasmodic, and diuretic. Fennel is used in treating glaucoma, galactagogue, and high blood pressure (Cioanca, Hancianu, Mircea, Trifan, & Hritcu, 2016). Research shows that fennel can increase the bioavailability of a variety of composites and drugs by reducing CYP2D6 and CYP3A4 enzymes in the liver. CYP2D6 and CYP3A4 metabolize (break down) a variety of prescription drugs, including antidepressants, antipsychotics, opioid pain relievers, antibiotics, blood pressure (beta-blockers), and cholesterol (statins) medications. The overall objective of this study was to present a succinct profile of fennel phytosomes to improve the oral bioavailability of fennel phytonutrients, provided the above reasons associated with the typically used types of fennel

dosages and the advantages possessed by modified forms of fennel phytosomes.

MATERIALS AND METHODS

2.1 Collection and extraction of seeds

The seeds of *Foeniculum vulgare* Mill. were collected from the accredited seed store from the University of Agriculture, Faisalabad. Seeds of *F. vulgare* were selected based on their medicinal uses related to our research but have low bioavailability. Seeds were collected and washed thoroughly. Dried the seeds under the shadow and then in the oven at 80°C. Seeds were ground and sieved into a fine powder carefully and in an isolated environment (Mali, Vyas, Dwivedi, & Joshi, 2014), and kept the powder in an airtight container. Afterward, a soxhlet apparatus method was used for the extraction of the extract. After extraction, the extract was filtered and concentrated with a rotary evaporator. The concentrated extract was stored in a freezer at 0°C for further analysis (Yazd et al., 2019).

2.2 Preparation of *F. vulgare* phytosomal complexes and calculation of *F. vulgare* content

F. vulgare and Soy Phosphatidylcholine were dissolved in 20 mL of absolute ethanol with quantities (500-2500 mg). The mixture was stirred for (120 - 360 min.) at a temperature not exceeding 25°C. The mixture was then isolated from the air with a vacuum evaporator. The dried residue was collected and put overnight in desiccators. The resulting *F. vulgaris* - phospholipid complexes were transferred to vials and stored at room temperature for further use. Weighed 5mg equivalent amount of complex was mixed with methanol in a volumetric flask to determine *F. vulgare* content at 288 nm λ max. Soy phosphatidylcholine was used as a blank sample solution. (Maryana, Rachmawati, & Mudhakir, 2016).

2.3 Preparation of *F. vulgare* phytosomes

Plant vesicles were formed through a thin-layer process using a vacuum rotary evaporator.

Dissolved the *F. vulgare*-SPC complex in absolute ethanol and integrated it into a 250 mL round-bottom flask. At 180 rpm, we connected the flask to the spinner and spin. The solvent was evaporated under reduced pressure at 60±20°C for 30 minutes to form a thin film on the edge of the flask. The cast membrane was distributed in phosphate-buffered saline (PBS, pH 7.4) at a temperature of 60±20°C. After approximately 15 minutes of hydration, the lipids swelled and peeled off the walls of the round-bottomed flask and formed vesicles. The plant body suspension was finally sonicated for 4 min in a probe sonicator with an amplitude of 60% and a switching time of 5 s. Store the plant suspension in the refrigerator for 24 hours before characterization (Maryana et al., 2016).

2.4 Entrapment Efficiency Calculation

Phospholiposome capture efficiency was calculated by measuring the amount of drug captured in the phospholiposomes. A sufficient amount of entrapped drug was transferred to determine the encapsulation efficiency of the drug in the plant body. Centrifuge the solution at 15,000 rpm for 15 min. The supernatant was collected after centrifugation, and the percentage of drug encapsulation of free drug was calculated spectrophotometrically (λ max = 288 nm). The encapsulation efficiency was determined according to the following formula:

$$EE \% = \frac{\text{Added drug} - \text{free drug}}{\text{Added drug}} \times 100$$

The added drug is the amount of drug added during the preparation of the plant body, and the free drug is the amount of free drug measured after centrifugation in the lower chamber of the culture tube (S. Jain, Ancheriya, Srivastava, Lal Soni, & Sharma, 2019).

2.5 Experimental design for the optimization of independent and dependent variables of phytosomal complexes

Factors (independent variables) that have a relatively strong effect on the rate of EE% and λ



max (dependent variable) were selected based on the preliminary experiment (Q.-y. Tan et al., 2012). These included: the concentration of *F. vulgare* (A mg), the concentration of soy phosphatidylcholine (B mg), and stirring time (C mins.). The response variables are entrapment efficiency (%) and λ max. According to the experimental design and the effects of the independent variables on the rate of EE% and λ max, all phytosomal complex formulations were prepared. An optimization process, i.e. external center composite design, was carried out. Briefly, the three variables were tested separately at five levels, and experiments were conducted on all 20 possible combinations (Q. Tan et al., 2012). All the data was countered to linear regression model (1) and quadratic model (2) (designed expert program).

The models are represented as follows:

$$Y = a_0 + a_1A + a_2B + a_3C \quad (1)$$

$$Y = b_0 + b_1A + b_2B + b_3C + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{12}AB + b_{13}AC + b_{23}BC \quad (2)$$

The intercepts representing the arithmetic mean response of the 20 runs were a_0 and b_0 , where Y

was the dependent variable (EE% and λ max), and the approximate coefficients for factor A were a_1 , b_1 . The key effects (A, B, and C) were the average consequence of changing one factor from its minimum to its maximum value at a time. The terms of interaction (AB, AC, and BC) showed how two variables were simultaneously changed and resultantly the response changed. To examine non-linearity, the polynomial terms (A^2 , B^2 , and C^2) were used. For some parameters, the optimized batch was selected among the solutions; all variables remain in range and trap efficiency as the limit that has been submitted to the design expert programmer. Table 2.5 shows the levels of independent variables and the composition of CCD batches.

Using tools from design experts, the effects of experimental designs were analyzed. For all phytosomal complexes, each response coefficient was examined at a 95 percent confidence level for its statistical analysis. P values less than 0.05 have shown a substantial difference, whereas the latter has a negligible difference (Liang et al., 2017; Q. Tan et al., 2012).

Table 2.5 Consideration of independent and dependent variables of *F. vulgare* Complexes in design used for optimization

Independent Variables	Levels					Dependent Variables
	1	2	3	4	5	
A^1 (mg)	500	1000	1500	2000	2500	Y= (%)
B^2 (mg)	500	1000	1500	2000	2500	Λ max
C^3 (mins.)	120	180	240	300	360	

1. A (concentration *F. vulgare* extract (mg))
2. B concentration of SPC (mg)
3. C stirring time (mins.)

2.6 Ultraviolet-visible (UV-Vis) spectroscopy

A sample being tested, such as *F. vulgare*, phosphatidylcholine soybeans, and *F. vulgare* phytosomes in a volumetric flask equal to **0.05 g** was dissolved in ethanol. Different dilutions (**5-10**) were made and then scanned with a UV

spectrometer in the wavelength range of 200-600 nm (Q.-y. Tan et al., 2012).

1.7 Fourier Transform Infrared Spectroscopy (FTIR)

Pellets were prepared by mixing the sample with dry crystalline KBr in the ratio of **1:100**. Grinded and pressed the mixture into a fine powder using an agate mortar before pressing into the KBr disk. Each KBr disk was scanned at a resolution **2 of 4 mm/s**, and spectra were collected in the



wavenumber region **4000-450 cm⁻¹** for each sample. The phospholipid (SPC) IR spectra, pure fennel, SPC, and fennel and fennel-SPC complex physical mixture were analyzed as contrasts (Maryana et al., 2016).

2.8 Antioxidant activity

2.8.1 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The DPPH radical scavenging activity assay was performed using the freshly prepared methanol solution of DPPH (**1ml, 0.1mM**) that was combined with various concentrations (**20-100ug/ml**) of *F. vulgare* phytosomes and ascorbic acid. The decrease in absorbance was estimated at **517nm** after **20 min** of further mixing. The standard use was ascorbic acid. Each sample's DPPH radical scavenging activity was measured as the inhibition percentage.

Percent DPPH radical activity inhibition = $[(A_0 - A_1)/A_0] \times 100$

Where **A₀** is the control absorbance and **A₁** is the sample absorbance of DPPH (Ahmed, Shi, Liu, & Kang, 2019)

2.8.2 Determination of reducing power

Different concentrations (**20-100 µg/ml water**) of *F. vulgare*, phytosomes, and ascorbic acid were mixed with **1 mL** of water, **2.5 mL** phosphate-buffered (**pH 6.6 and 0.2 M**), and **2.5 mL 1% C₆N₆FeK₃**, respectively. Incubated the mixture for 25 minutes at 50°C. Centrifuged mixture at **3000 rpm** for 15 min after adding **10% of 2.5 ml** Trichloroacetic acid. Add **2.5 mL** of double-distilled water and **0.5 mL** of ferric chloride (0.1%) in removed supernatant. Measured the absorbance at **700 nm** of each solution (Zahra, Jahan, Nosheen, & Khalil-ur-Rehman, 2011).

2.9 Determination of ACE inhibitory activity

ACE was extracted from rabbit lungs following the procedure of (KIM, Dae-Hyoung, Jong-Soo, CHUNG, & JEONG, 2004; Luna et al., 2009; Vermeirssen, Van Camp, & Verstraete, 2002) with some changes. After this borate buffer (**50µl**) was

mixed with an ACE solution of **100µl/ml (50µl)** and incubated for 10 minutes at 37°C. After incubation, **150µl** substrate solution was mixed with the reaction mixture and kept for incubation at 37°C for 80 minutes. Substrate solution was made by dissolving Hip-His-Leu (**8.3mM**) into PO₄ buffer. After incubation for 80 minutes, **250mcL** of 1M HCl was added to stop the reaction. Ethyl acetate (**1500µl**) was added to the reaction mixture to extract hippuric acid formed during the reaction and centrifuged for 15 minutes at 4000rpm. After centrifugation 1.5ml of supernatant was pipette out and shifted in a separate beaker. The beaker was covered with a net and set aside overnight to achieve a dried solution. In the dried solution, 1ml of distilled water was mixed and absorbance was noted at **228nm**. The blank reaction was prepared using the same method, except that HCl was added before the substrate addition. The percentage inhibition of aqueous extract of plant and standard (captopril) was determined by the same procedure described above except **50µl** of buffer was replaced with the same amount of plant extract (**3mg/ml**). The blank sample was prepared in the same manner as the blank reaction was prepared by changing the buffer with plant extract (Belović, Ilić, Tepić, & Šumić, 2013; Cushman & Cheung, 1971).

The ACE inhibition percentage was determined using the following formula:

ACE Percentage of inhibition = $[(A-B)-(C-D)/(A-B)] \times 100$

Where, in the presence of ACE, **A** represents absorbance, **B** is blank reaction absorbance, **C** is the absorbance in the presence of ACE and inhibitors, and **D** is blank reaction absorbance.

RESULTS AND DISCUSSION

3.1 Optimization of Independent and Dependent Parameters for the Formulation of *F. vulgare* Complexes

Complexes arranged following twenty results devised by CCD three limiting factors. All five



levels of the three limiting factors were concentration of *F. vulgare* (**500mg-2500mg**), conc. Of SPC (**500mg, 2500mg**), and stirring time (**120- 360mins**). Contents of *F. vulgare* were determined by UV- visible spectrophotometer at 288nm λ max and entrapment efficiency (%) was calculated. The responses of designed experiments by CCD are shown in Table 3.1. The response variable (%) was varied from **95** to **21.05** and λ max from **290nm** to **253nm**. The optimum entrapment efficiency and λ max were devised and examined with experiment design 7.0.0. A quadratic model was used to determine the optimal conditions of limiting factors.

CCD evaluated that, the response λ max of *F. vulgare* phytosomal complexes varied from 253nm-290nm which shows showing the measure in drug concentration from 500mg- 1500mg

enhanced the entrapment efficiency of complexes. Further, an increase in *F. vulgare* concentration decreased the entrapment efficiency (%) of phytosomal complex of *F. vulgare*. Entrapment efficiency (%) was increased with the increase in the concentration of SPC from **500mg** to **2500mg**, same trend was observed for stirring time. Entrapment efficiency was increased from **120** to **240 minutes**. And decreased with an increase in stirring time to **360mins** while further increase in time showed an increase in entrapment efficiency again.

The highest entrapment efficiency of *F. vulgare* phytosomal complex (**95%**) was observed at **1500mg** of *F. vulgare* conc., **1500mg** of SPC, and **240mins**. stirring time. The lowest (%) was observed at **3181.79mg** *F. vulgare*, **1500mg** SPC, and **240 minutes** of stirring time (Table 3.1).

Table. 3.1 Response of experiments designed by CCD.

Sr. No.	Independent variables			Responses	
	<i>F. vulgare</i> conc. (mg)	SPC conc. (mg)	Stirring time (mins)	(R1) λ max (nm)	(R2) Entrapment efficiency (%)
1	1500	1500	441.82	264	80
2	1500	3181.79	240	259	87
3	1500	1500	38.18	259	92
4	500	500	360	265	60.01
5	1500	-181.79	240	279	0
6	1500	1500	240	260	77.01
7	1500	1500	240	255	76
8	500	2500	120	266	86.03
9	-181.79	1500	240	258	0
10	3181.79	1500	240	270	21.05
11	1500	1500	240	257	71
12	2500	500	120	265	31
13	2500	2500	360	261	58.04
14	2500	500	360	290	28.07
15	500	500	120	265.5	39.03
16	1500	1500	240	265	81
17	2500	2500	120	259.5	60
18	500	2500	360	253	70
19	1500	1500	240	263	75
20	1500	1500	240	258	95

CCD= Central Composite Design

SPC= Soy Phosphatidylecholine

3.1.1 Selection of Model, Regression Analysis, and ANOVA for Response Surface Quadratic Model of *F. vulgare* phytosomes



It is provided that the quadratic model is the best fit for the responses studied, namely mean, EE%, and λ max. The quadratic equation was generated for the responses.

Quadratic equation in terms of coded factors:

$$(F. \text{vulgare } \lambda \text{ max}) (R1) = +259.65 + 3.38A - 5.83B + 1.57C - 2.87AB + 5.00AC - 4.50BC + 1.63A^2 + 3.40B^2 + 0.75C^2 \quad (1)$$

$$(F. \text{vulgare entrapment efficiency}) (R2) = +78.87 - 3.12A + 19.20B - 1.47C + 0.25AB - 1.23AC - 4.50BC - 22.30A^2 - 10.64B^2 + 4.38C^2 \quad (2)$$

The above equations have controlled results of a single limiting factor or a combination of limiting factors on responses R1 and R2. Positive coefficient values have indicated positive interactions of the factors and their impact on R1 and R2, while negative values have indicated the

interference of the parameter on the overall response. From Equation (1), it is obvious that A and B linear main effects were statistically significant due to p-value < 0.01. However, the stirring time(C), had no significant effect due to > 0.01 p value. The positive effect of factors A and B showed that an increase in their rate also increases the response λ max. All the interaction terms (AB, AC, and BC) and quadratic effect (B^2) were also significant statistically and had positive effects except (AB and BC) Table 3.2 has an explanatory and response variables relationship which can be easily seen by RSM. Plots were generated by a design expert, as shown in Fig. 3.2. High levels of A and C and low levels of B were the best conditions for high λ max.

Table 3.2. Generalized Quadratic model frame for statistically significant test terms

source	A	B	C	AB	AC	BC	A ²	B ²	C ²
	λ max								
Coefficient	3.38	-5.83	1.57	-2.87	5.00	-4.50	1.63	3.40	0.75
F value	17.8	52.94	3.83	7.54	22.8	18.47	4.38	19	0.92
P value	0.0018	<0.0001	0.0789	0.0206	0.0008	0.0016	0.0628	0.0014	0.3596
	Entrapment efficiency %								
Coefficient	-3.21	19.20	-1.47	0.25	-1.23	-4.50	-22.30	-10.64	4.38
F value	0.62	23.65	0.14	2.30	0.057	0.76	33.66	7.67	1.3
P value	0.4484	0.0007	0.7169	0.9627	0.8164	0.4031	0.0002	0.0198	0.2809

From equation (2), the results are represented in Table. 3.2, terms (B and A², p < 0.01) is statistically significant. B linear term had a positive correlation with response EE% and approaching maximum value due to the opposite relation between linear and quadratic effects (B and B²). Quadratic and linear effects of (A, C, A², and C²) had negative correlations with the response limiting factor (EE %). Interaction terms (AB, AC, and BC) suggest a complex relationship with response EE%.

Design expert plots for the relationship between response variable EE% and independent limiting factors are shown in Fig. 3.3.

Values of R², SD, coefficient estimate, and ANOVA for the responses are shown in Table 3.3. The results of ANOVA and R² predicted that the model was significant for response limiting factors.

Table. 3.3 Analysis of variance of λ max (R1) and Entrapment efficiency (%) response surface quadratic model of *F. vulgare* phytosomes.

Source	SS	DF	MS	F	p-value	R ²	SD	CV%
				λ max				
Model	1274.59	9	141.62	16.15	<0.0001 Significant	0.9356	2.96	1.12
Residual	87.71	10	8.77					
Total	1362.3	19						
				Entrapment Efficiency%				
Model	14371.13	9	1596.79	7.5	0.0021 Significant	0.8709	14.59	5.5
Residual	2129.84	10	212.98					
Total	16500.97	19						

3.1.2 Diagnostic Plots

The diagnostic plot of the two responses (R1 and R2) calculated by the quadratic model is shown in Fig. 3.1.

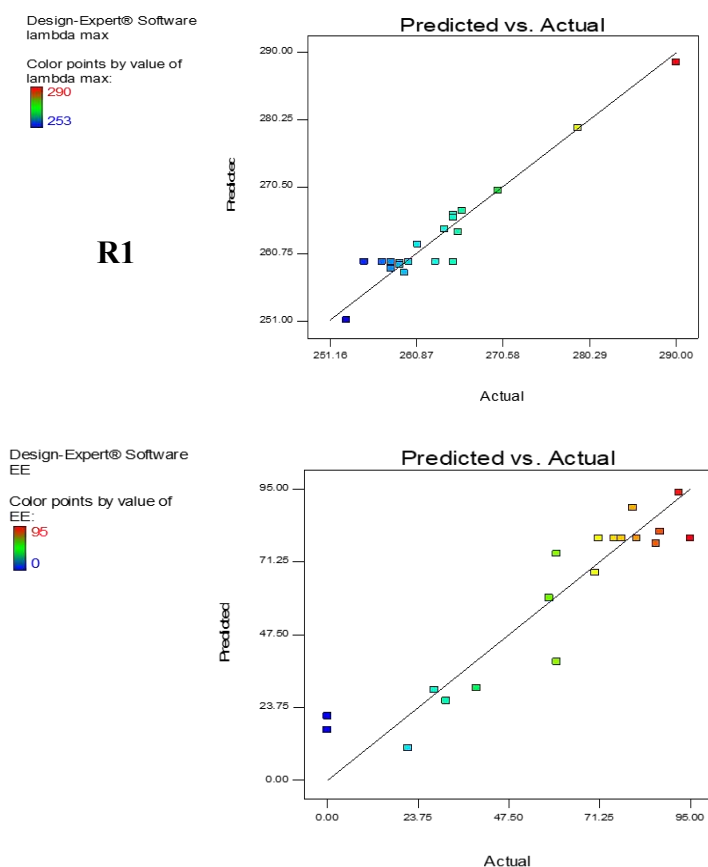


Figure. 3.1 Predicted vs. Actual plot for R1 and R2.

3.1.3 3D Response Surface Plots

Dependent and independent limiting factors are more illuminated by formulating response surface plots. Designed 3D RS graphs for statistically analyzed limiting factors (responses) on evaluated parameters shown in Fig. 3.2 and Fig. 3.3. A 3D response surface plot was determined due to the effect of limiting factors (plant extract, SPC

concentration, stirring time) on the response λ max and encapsulation efficiency (R1 and R2).

Extrapolating from the present results, the amount of plant extracts, SPC, and stirring time had an optimized role. All the independent limiting factors (extract, SPC, stirring time) had a greater effect on the λ max (response) of phospholiposomes

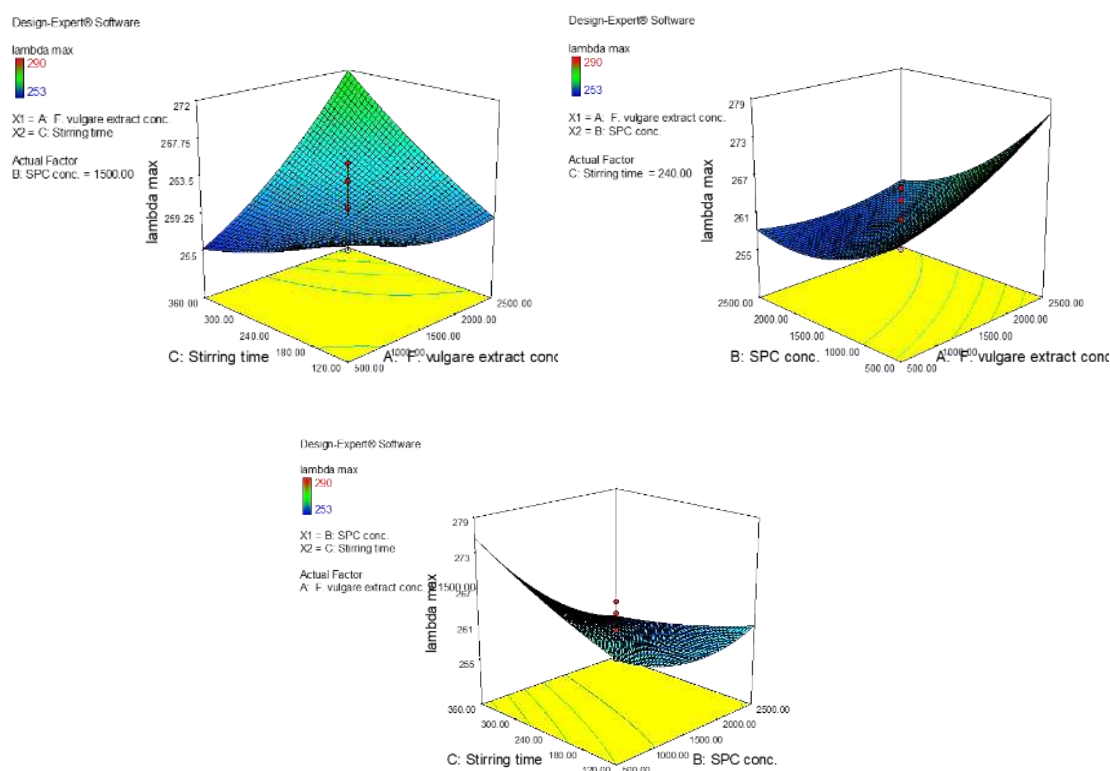


Figure. 3.2 3D graph of response (R1) lambda max interaction with (a) extract/ SPC, (b) stirring time/ SPC, and (c) stirring time/ extract

The RSM has shown that not only SPC concentration was enough to increase the entrapment efficiency (%) of the complex. Stirring

time has also enhanced EE (%). EE (%) enhancement is due to the assembling and physical interactions of phospholipids-F. vulgare amounts.

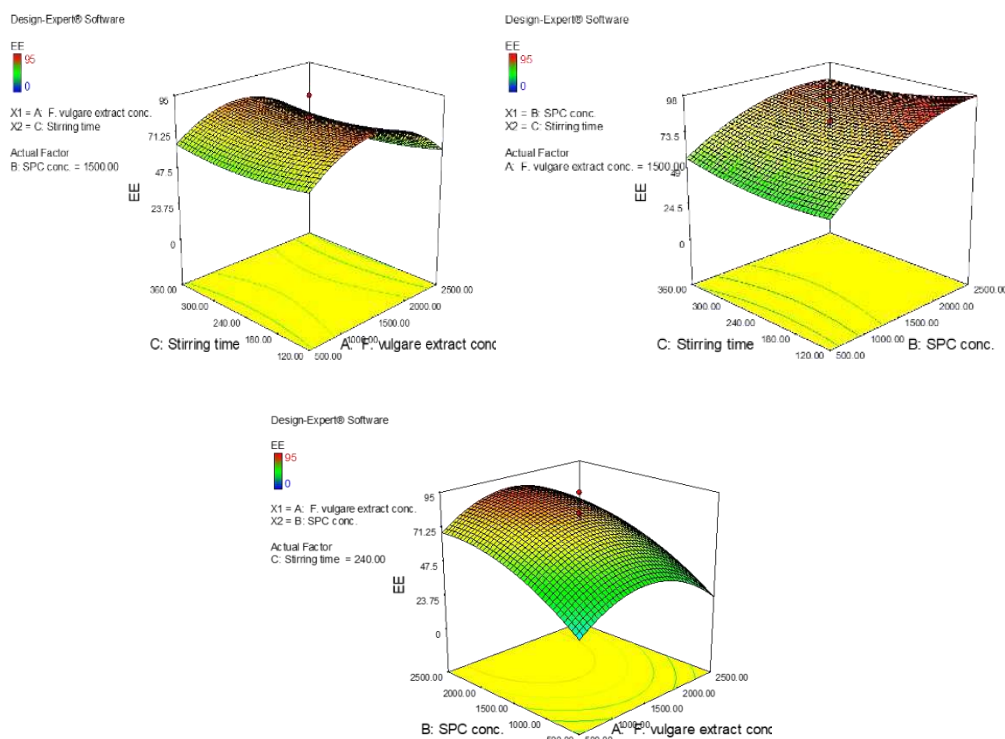


Figure. 3.3 3D graph of response (R2) EE interaction with (a) stirring time/ SPC, (b) extract/SPC, and (c) stirring time/extract

All independent limiting factors were designed to impress the responses remarkably. Soy phosphatidylecholine concentration has increased entrapment efficiency (%) remarkably. At low to average stirring time (120-240 min.) entrapment efficiency (%) was enhanced due to the assembling of phospholipid-*F. vulgare* molecules and physical interactions develop between them. Stirring time (360mins.) has reduced entrapment efficiency (%) again due to the breakdown of physical interactions, however further increase in stirring time, has increased entrapment efficiency.

3.2 Characterization

3.2.1 UV-Visible Spectroscopy

In this study, *F. vulgare*, phospholipid, and *F. vulgare* phytosome were scanned in the range of **200-600 nm**. In the UV-visible spectra of *F. vulgare*, a sharp band was observed at **247nm** and phospholipid showed a sharp absorption band at **270 nm**. The UV-visible spectrum of *F. vulgare* phytosome showed a sharp absorption band at **259nm**. The UV spectrum of *F. vulgare*

phytosome was quite similar to the spectra of *F. vulgare* and phospholipid. Their pictorial evidence is in Fig. 3.4. Physical interaction development between the two factors (extract and SPC) may be much weaker to be noticed (Q.-y. Tan et al., 2012).

UV-Visible spectra of *F. vulgare* extract, Phytosomes, and Phospholipids

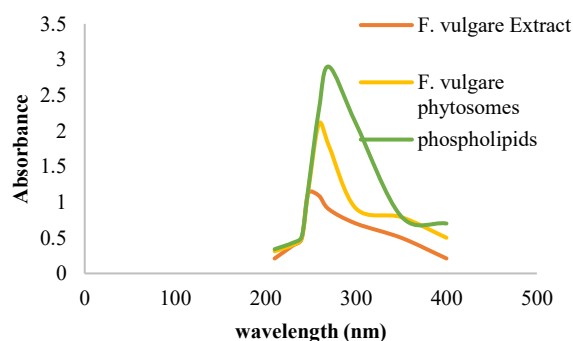


Figure 3.4 UV-Visible spectrum of *F. vulgare*, *F. vulgare* phytosomes, and Phospholipids

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis has shown the following functional groups are present in *F. vulgare* extract, phytosomes, and SPC which are enlisted in Table. 3.4

Table. 3.4 Functional groups present in *F. vulgare* extract, phytosomes, and SPC and their peak values

Sr. No.	Functional groups	SPC	<i>F. vulgare</i> phytosome	<i>F. vulgare</i>
1	$\nu(\text{C=O})$	1734.06	1732	1742
2	$\nu\text{s}(\text{CH}_2)$	2922.05	2924	2921
3	$\nu(\text{P=O})$	1232.60	1237	-----
4	$\nu(\text{P-O-C})$	1080.07	1041	-----
5	$\nu\text{as}(\text{CH}_2)$	-----	2854	2851
6	$\nu(\text{C-C-N})$	966.26	-----	-----
7	$\nu(\text{O-H})$	-----	3454-3124	3296
8	$\nu(\text{CH}_3)$	1462.87	1460	-----
9	$\nu(\text{C=CH})$	-----	721	-----
10	$\nu(\text{C-H})$	-----	1160	1027
11	$\nu(\text{C=C})$	-----	-----	1597

These observations indicated that some chemical interactions were developed between *F. vulgare* and phosphatidylcholine contents. In *F. vulgare*, IR spectrum has bands at 3926cm^{-1} (OH), 2921cm^{-1} s(CH₂), 2851cm^{-1} as(CH₂), 1742cm^{-1} (C=O), 1597cm^{-1} (C=C) and 1027cm^{-1} (C-H) (Dager, Uchida, Maekawa, & Tachibana, 2019). In the phospholipid (SPC) spectrum, absorptions appeared at 2922.05cm^{-1} (CH₂), 1734.06cm^{-1} of (C=O), 1462.87cm^{-1} (CH₃), 1232.60cm^{-1} (P=O), 1080.07cm^{-1} (P-O-C) and 966.26cm^{-1} (C-C-N) (Q. Tan et al., 2012). The spectrum of phytosome was slightly different from extract and SPC. Bands at 1597cm^{-1} and 966.26cm^{-1} were not present or covered by extract and SPC in complex or phytosome. Bands or peaks at 1742cm^{-1} and 1734.06cm^{-1} were decreased to (1732cm^{-1}) closely the same region of SPC. Absorptions at 2921cm^{-1} and 2922.04cm^{-1} increased to (2924cm^{-1}) also close to the SPC region (Table 3.4, Fig. 3.5). The FTIR results confirmed that chemical and physical interactions occurred between *F. vulgare* and phospholipid for the synthesis of *F. vulgare* phytosome (Q.-y. Tan et al., 2012). Absorption spectra of SPC and *F. vulgare* extract were taken

from Dager *et al* and Tan *et al* findings (Dager et al., 2019; Q. Tan et al., 2012) for discussion and comparison. Phytosomes showed both of the peaks of plant extract and phospholipid with slight differences. The peaks of the spectrum present in both extract and phytosomes were shifted to the left side in phytosomes as compared to extract (Table 3.4). FTIR spectrum of *F. vulgare* phytosomes has been showing the peaks at $3454\text{--}3124\text{cm}^{-1}$ (OH), 1237cm^{-1} (P=O stretching), and 1041cm^{-1} (P-O-C).

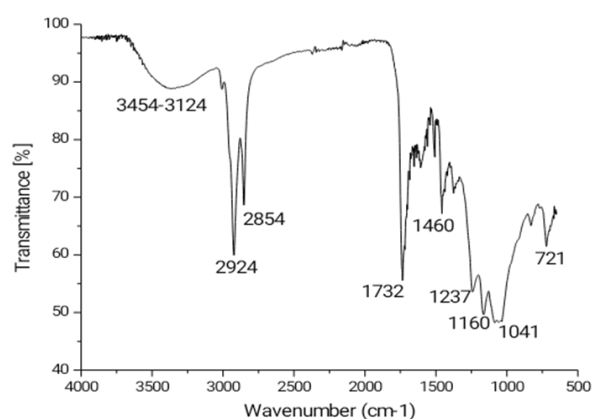


Figure. 3.5 FTIR spectra of *F. vulgare* Phytosomes

3.3 Antioxidant Activity of *F. vulgare* Phytosome

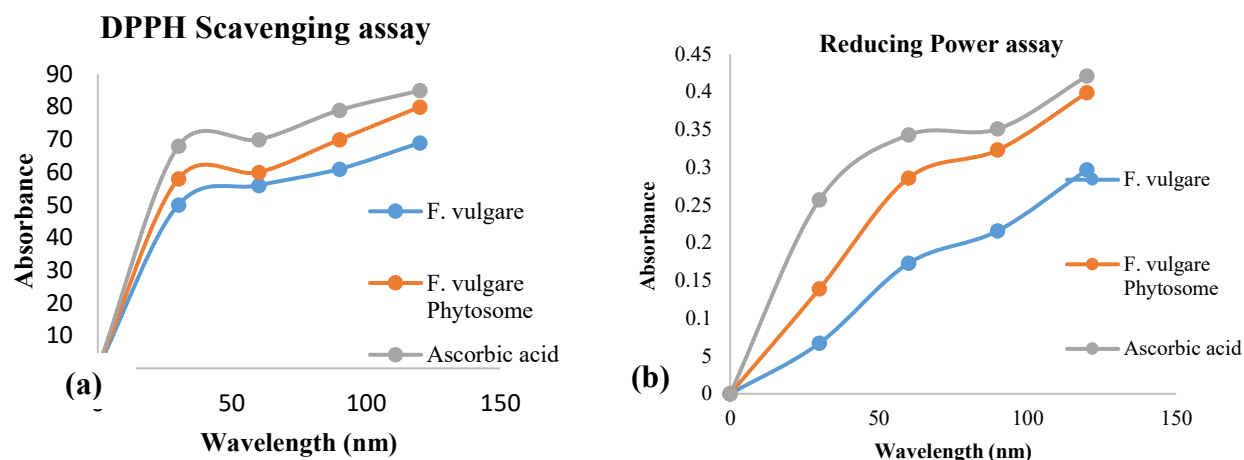


Figure. 3.6 (a) 2, 2- Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging and (b) Reducing Power Assay
 This study shows *F. vulgare* phytosomes have a higher %age inhibition than crude. The maximum %age inhibition of *F. vulgare* phytosomes noted was 80% in DPPH scavenging and 0.399 in reducing power assay which is shown in Fig. 3.6 (a) and (b), respectively. Ascorbic acid was used as standard. Hence it shows *F. vulgare* phytosomes radical scavenge is more than crude plant extract (69%, 0.297), which is closely related to ascorbic acid.

3.4 ACE Inhibition Activity

Table. 3.5 ACE inhibition of plant extract, phytosomes, and standard values

Concentrations	% ACE Inhibition		
	Plant extract	Standard	Phytosomes
0.5mg/ml	50%	53%	68%
1.5mg/ml	52%	57%	71%
2.5mg/ml	56%	59%	74%

Captopril was taken as a standard for checking ACE enzyme activity. As shown in the graph, captopril has a maximum %age inhibition which is **59%** at high (**2.5mg/ml**) concentration and **53%** at low concentration (**0.5mg/ml**). Phytosomes gave **74%** at high concentration (**2.5mg/ml**) and **68%** at low concentration (**0.5mg/ml**), and crude shows **56%** at high concentration and **46%** at low

concentration (Fig. 3.7, Table. 3.5). Overall study of ACE inhibition revealed that fennel phytomes have more capability to decrease the oxidative stress and act as anti-hypertensive drug than simple /crude extract of fennel seeds. Secondary metabolites present in fennel play a role in ACE inhibition especially trans-anethole gave good results in ACE inhibition (Chaudhary et al., 2013).

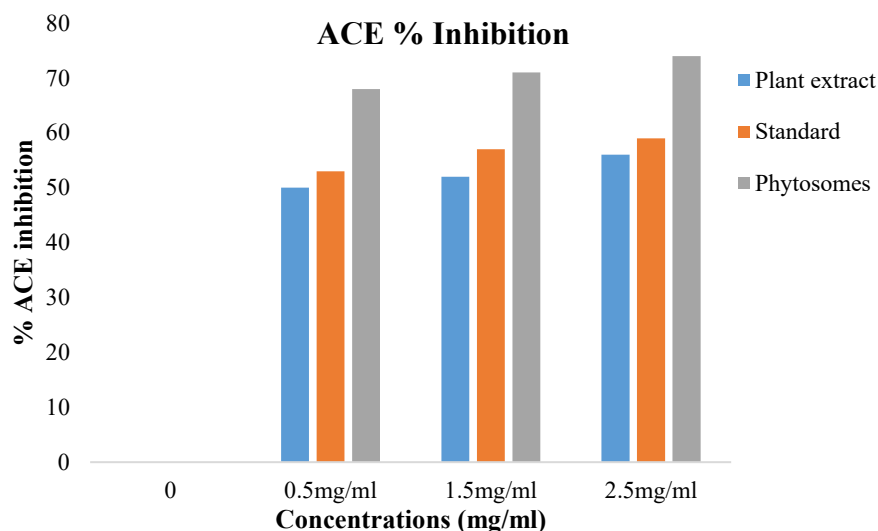


Figure. 3.7 ACE inhibition of *F. vulgare* phytosome, standard, and crude

CONCLUSION

Novel drug delivery systems such as phytosomes have emerged as one of the loading methods for improving the bioavailability of poorly soluble bio-actives and extracts. *F. vulgare* contains hepato-protective flavonoids but with low bioavailability. This study was done to formulate the *F. vulgare*-loaded phytosome to improve the bioavailability and ACE inhibition potential of *F. vulgare* with better therapeutic potential. Firstly, the *F. vulgare* phytosomal complexes were prepared by solvent evaporation method then these complexes were incorporated into phytosome vesicles prepared by thin layer hydration technique. *Foeniculum vulgare* phytosomes were optimized by using response surface methodology (RSM). Independent variable's effect (fennel extract, phosphatidylcholine, and stirring time) with their interactions, had been evaluated and modeled. The highest entrapment efficiency (%) was confirmed at 1500mg/1500mg (fennel extract/phosphatidylcholine) amounts, 240 (min) stirring with 259 (nm) λ max. Fennel phytosomes show the highest antioxidant and ACE inhibition values than crude extract which shows that phytosomes enhanced the bioavailability and ACE inhibition potential of fennel secondary metabolites. Results obtained by this research

could potentially be exploited as an antihypertensive drug. Results obtained from this study conclude that fennel could be used as a natural product in better-advanced ways of drug delivery to cure diseases related to hypertension and many others.

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