Pharmacogn. Res., 2023; 15(3):578-590.
https://www.phcogres.com

Original Article

Effect of Cholesterol and Different Solvents on Particle Size, Zeta Potential and Drug Release of Eucalyptus Oil Phytosome

Sharad Visht*, Sana Sirwan Salih

Department of Pharmaceutics, Faculty of Pharmacy, Tishk International University, Erbil, KRG, IRAQ.

ABSTRACT

Background: Herbal extracts show poor absorption and bioavailability but their complex with phospholipids i.e., phytosome improves this major problem. **Aim:** The aim of research was to examine the effect of cholesterol and different organic solvents on particle size and zeta potential of eucalyptus oil phytosome. **Materials and Methods:** Totally six batches of phytosomes were prepared using three solvents chloroform, dichloromethane, acetone, with and without addition of cholesterol. Phytosome were evaluated for yield, FTIR, partial size and zeta potential, drug entrapment and drug release, drug release kinetics and stability studies. **Results:** The yield, density refractive index and maximum absorbance (λ_{max}) of eucalyptus oil was found to be 1.65±0.74%, 0.9928 g/cm^3 (24.2°C), 1.3613(24.6°C), and 297.733 nm respectively. There was no drug excipient interaction. The yield varied from 85.14±0.74 to 87.14±0.74%, particle size varied from 71.76±0.63 to 197.36±0.53 nm, zeta potential varied from 15.3±0.27 to-28.2±0.26 mV and entrapment efficiency varied from 57.45±0.35 to 67.34±0.52 respectively. The *in vitro* drug release varied from 87.26±0.63 to 71.35±0.63% up to 300 min (5 hr) and batch-A was selected as best formulation that showed Peppas Korsmeyer as the best fit model with R^2 value 0.9422 and mechanism of drug release was Fickian Diffusion (Higuchi Matrix). The stability studies showed 99.14±0.25 to 99.55±0.52% drug content on 28th day. **Conclusion:** The particle size of phytosomes was increased on addition of cholesterol. The acetone showed the smallest particle size and chloroform showed the biggest particle size that indicated that molecule diameter and molecular weight affects the solubility and assembly of phospholipid/ cholesterol in solvent in order to prepare particulate drug delivery system.

Keywords: Eucalyptus, Phytosomes, Evaluation, Particle size, Zeta potential.

Correspondence:
Prof. Sharad Visht
Department of Pharmaceutics, Faculty of Pharmacy, Tishk International University, Kurdistan Region, IRAQ.
Email id: sharad.visht@tiu.edu.iq

Received: 14-12-2022;
Revised: 11-01-2023;
Accepted: 03-03-2023.

INTRODUCTION

The phytomedicine's possess a lot of therapeutic benefits and thus used for health maintenance subsequently since prehistoric periods.[1,2] Flavonoids and terpenoids are one of the most important bioactive photochemical, highly polar in nature and thus poorly absorbed in lipids[problem in permeation] and leads to poor bioavailability.[3] Various methods and approaches have been reported for the improvement of the bioavailability like use of solubility/bioavailability modulators, structure modifications, entrapment of phytoconstituents with lipophilic carriers.[4] Among various approaches, one approach is the phytosome technology.[5] This technology was discovered by Indenato combat the issue of poor bioavailability of phytoconstituents.[6] The phytosome is derived from "Phyto" i.e, plant and "some" i.e., cell resembling structure.[7]

Phytosomes are the one of the promising, particulate drug delivery system that is used for novel/ Targeted Drug Delivery Systems (NDDS/ TDDS).[8] Phytosome is an advanced ways to deliver the water soluble phytoconstituents or herbal extracts either complexed with phospholipids (flavonoid/ terpenoid based compounds in herbal extracts binds with phosphatidylcholine) or surrounded by phospholipids that shows better pharmacokinetic and pharmacodynamic profile as an improved absorption and bioavailability of phytoconstituents, specially poly-phenolics.[9] Phytosome is a complex of phospholipid with herbal molecules formed by molecular-level association.[10]

The Phosphatidylcholine (PC) are the commonly used lipid phase to prepare the phytosomes.[11] Phospholipids (PC) are the major component of all cell membranes.[12,13] It forms a lipid bi-layer structure on hydration.[14-16] Phosphatidylcholine is an amphipathic molecule and structurally it bears a positive charged head and two neutrally charged tail that turn this molecule well absorbable orally as it favours both water and lipid environment.[17] The oxygen atom acts an electrophilic and the nitrogen atom acts as electron donor in the phosphate group. The phosphate group of phosphatidylcholine i.e., polar

DOI: 10.5530/pres.15.3.061

head of PC (choline) and hydroxyl group of phytoconstituents interacts by formation of H-bond results in better physical stability with enhances drug absorption/ bioavailability of hydrophilic polar herbal molecules,[9,18,19] for better therapeutic benefits, improvement in efficacy and decrease in toxic side effects of drug as phytosomes.[20,21] Any phytoconstituents with an active hydrogen atom like -OH, -COOH, -NH, =NH, -NH_2 can synthesized into esters (with or without use of spacer chain) to the –OH group of a PC molecule.[19] These formed esters are amphiphilic agent that facilitates the crossing of phytosomes though the cell-membrane barrier due to non-polar behaviour of synthesized ester molecule.[19,22] The destruction of water soluble herbal molecules like phenolics, glycosides, terpenoids and flavonoids by gastric secretion and microflora of gut can be prevented by formulating their phytosomes.[9,23]

The phytosomes involve one phospholipid and one drug where hydrophilic phytoconstituents is fixed through chemical bonds (H-bond) to the hydrophilic head of the phospholipid (complex is soluble in both aqueous/ non-aqueous phase).[24-26] In case of liposomes, the active ingredients are water-soluble and placed at center hydrophilic cavity or in between the hydrophobic layers of the fatty acid chains.[27,28] Several phytosomes have been formulated and marketed using herbs like *Ginkgo biloba*, grape seed, *Crataegus, Silybum marianum, Camellia sinensis, Panax ginseng*. Generally the phospholipid complexes with daidzein, salvianolic acid B, clarithromycin, rutin, luteolin, curcumin, silybin, valproic acid using non-aqueous solvents offers several outstanding advantages over pure drug like improved drug absorption to selected tissue specially in anticancer activity with low cyto-toxicities).[29] Silybin as phytosome formulation showed approximately seven times greater absorption of silybin as compared to the milk thistle extract.[30]

Eucalyptus tree (*E. globulus*) belongs to Family-Myrtaceae.[31] It is a native of Tasmania and Australia. It is cultivated in South Franc, Spain, Brazil, Portugal, and India. Eucalyptus oil is a pale-yellow/ colorless liquid with a strong, camphor like flavor with cooling sensation. It must containat least 70% cineole of the total weight of the eucalyptus oil.[32,33] It brace varity of applications like expectorant, antimicrobial (antifungal, antibacterial), antioxidant, antiseptic, upper respiratory infections (URI), some skin disorders, malaria, flavoring, anti-scabies, insect/ mosquito repellent repellent, insecticidal.[34-37] Its essential oils acts as insecticidal on *Lutzomyia longipalpis*.[38,39] It is used as a stimulant expectorant, in asthma and in the treatment of chronic obstructive bronchitis. Due to poor aqueous solubility, its absorption/ bioavailability is less and several approaches have been employed to improve it.[40] The objective of the study was to examin the effect of different solvents (Table 1) and cholesterol on size, zeta potential, drug release and stability of eucalyptus oil phytosomes.

MATERIALS AND METHODS

Eucalyptus oil, the phospholipid was Soya lecithin E322 [Manufacturer: Kimyaciniz], cholesterol, dextrose anhydrous [Analytical Reagent], chloroform, dichloromethane, and acetone. All of the chemicals used were of the analytical grades. The fresh leaves of eucalyptus tree were procured in morning time form Sami Abdulrahman Park, Erbil on 20/October/2021 and sent for authentication. Leaves were dried in shade and crushed into powder to extract volatile oil using Clevenger apparatus.

Extraction of Eucalyptus oil

The fresh leaves of eucalyptus leaves were hydro-distilled to obtain the volatile oil using Clevenger apparatus. Accurately weighed amount (5 kg) fresh leaves were packed into 5 L Round Bottom Flask (RBF) continued the water up to ¾ volume. The assembly was run for 4 hrs and extracted oil was collected in volumetric flask.[41]

Purification of extracted eucalyptus oil

Extracted oil was mixed with Dichloromethane (DCM) to separate volatile oil from fat and lipids. The DCM was separated using separating funnel. The volatile oil was separated by placing DCM fraction in a flask, connected with vacuum rotary evaporator (Rota vapor). The volatile oil was collected in volumetric flask.[42]

Characterization of extracted eucalyptus oil

Various parameters like yield of oil, density of oil, refractive index of oil, UV-visible spectroscopy, FTIR and drug-excipient interaction were evaluated.

Yield

The yield (% v/w) was calculated as per following formula:[43,44]

$$Yield\ of oil(\%) = \frac{Volume\ of oil\ obtained}{Mass\ of\ leaves} X\ 100$$

Density

The Densito-pro machine was used to measure the density of extracted oil.

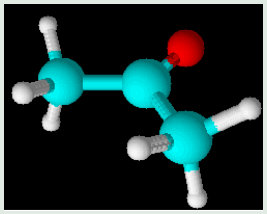
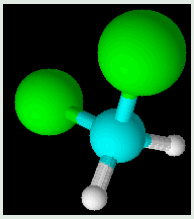
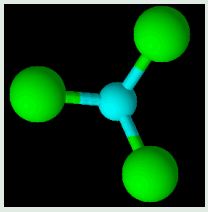
Refractive index

Mettler Toledo 30px tube was used to measure refractive index. The oil drops (2-3) were placed on the platform and result was displayed on monitor.

UV-visible Spectrophotometery

The stock solution (1000 µg/mL) was prepared in chloroform and different dilutions were prepared. Randomly one sample was scanned for maximum absorbance (λ_{max}) and further dilutions

Table 1: Solvents to prepare phytosomes.

Properties	Acetone	Dichloromethane	Chloroform
Structure			
Molecular weight [g/mol]	58.08	84.93	119.38
Molecular diameter (Å)	6.16	6.28	6.9
Polarity Index	5.1	3.1	4.1
Dielectric constant	20.7	9.08	4.81
Type of solvent	Aprotic	Aprotic	Aprotic

were scanned to measure the absorbance and calibration curve was plotted absorbance vs concentration.[45]

Fourier transform inferred spectroscopy [FTIR] of eucalyptus oil

The FTIR study was performed using FTIR (Shimadzu, IRAffinity-1S). FTIR is used for the identification of different type of bonds and functional groups in a pure molecule or a mixture. The FTIR of curcumin and amaranth was also performed. The sample was place on IR window and scanned from range 500-4000 cm^{-1}.[46,47]

Purification and drying of Soya lecithin E322 [Phospholipid]

The ethyl acetate was added to soya lecithin to remove the stickiness and to convert semisolid soya lecithin in to powder soya lecithin. Accurately weighed soya lecithin (20 g) was mixed ethyl acetate (200 mL) in a beaker (500 mL) and sonicated for 10 min. and stirred for 1 hr at 100 rpm. The ethyl acetate layer was decanted in triplicate. The soya lecithin residue was separated at bottom and dried to get soya lecithin powder and stored in airtight container at cool place.[48]

Characterization of purified soya lecithin E322 [Phospholipid] by FTIR

The FTIR of purified soya phospholipid was determined as discussed as previous.

Drug-excipient interaction as determined by FTIR

The eucalyptus oil, soya phospholipid, dextrose, cholesterol, was scanned for FTIR to determine the interaction between drug and excipients as discussed in previous section.[49]

Development of Eucalyptus oil phytosomes

The eucalyptus oil phytosome were prepared by using reflux method using composition mentioned in Table 2. The mixture of eucalyptus oil and phospholipid were kept in three 200 mL beaker contained solvents acetone, dichloromethane, and chloroform individually refluxed for 2 hr. By reflux, chemical bonds were developed between eucalyptus oil and soya phospholipid. Then prepared 3 batches of phytosomes were divided into two groups. In first group dextrose was added as re-hydrant and cholesterol was not added using same 3 solvents individually. In another batch dextrose was added as re-hydrant and cholesterol was added using same 3 solvents using sonication and magnetic stirrer at 50 rpm. Finally total six batches were prepared using three different solvents i.e., chloroform, dichloromethane, acetone as shown in Figure 1.[50]

Evaluation of phytosomes

The yield, particle size, zeta potential, entrapment efficiency, drug content, FTIR, *in vitro* drug release, drug release kinetics and stability study were examined.

Determination of % yield

The yield was calculated using below mentioned formula:[51]

$$[\%]\,Yield\,(\%) = \frac{Practical\ yield}{Theoretical\ yield} X\ 100$$

Particle size

The "Llitesizer 500" (Anton Paar GmbH Company) was used to measure the particle size of liposome. Accurately weighed phytosome of eucalyptus oil were placed in 2 mL of water and sonicated for 5 min and further analyzed using single use plastic curette in triplicates.[51,52]

Zeta potential measurement

The greater stability of any dispersion is considered based on high electrostatic repulsion between the particles. Zeta potential value > +20 mV or < -20 mV indicates the good physical stability of a dispersion. A small amount of sample form each batch was diluted with using deionized water and sonicated for 1 min. The sample was filled into the omega corvettes to measure the zeta potential.[52]

Entrapment efficiency

The dilution was prepared (1/10 mL methanol) and further centrifuged for 30 min. At 18,000 rpm to separate the supernatant and pellet. The free eucalyptus oil was calculated by UV/VIS-Spectrophotometery at 297.733 nm to determine the total mass of eucalyptus oil. The eucalyptus oil phytosome suspension equivalent to 0.1 mL was diluted using methanol. The volume was adjusted to 10 mL. The entrapment efficiency was calculated using formula given below:[53]

$$\text{Entrapment efficiency [\%]} = \frac{\text{Total drug} - \text{Free drug}}{\text{Totaldrug}} X\ 100$$

FTIR (Shimadzu Affinity) spectral data were taken to confirm the structures and chemical stability of phytosome. Samples were placed on IR window scanned in the range between 4000-500 cm^{-1}.

In vitro drug release

The *in vitro* release study was performed using a Franz diffusion cell. The phosphate buffer solution pH 7.4 was used as dissolution medium. The cellulose nitrate membrane was placed in between donor and receptor compartment of Franz diffusion cell. The 3 mL volume of the phytosome suspension (equivalent to 0.2 g Eucalyptus oil) was pipette into donor compartment. Diffusion medium (20 mL) maintained at 37±1°C. The membrane just touches the receptor medium surface. A magnetic bar continuously stirred at 100 rpm in the diffusion medium. Each of 3 mL volume were withdrawn at required time intervals. The fresh 3 mL volume of buffer was added receptor medium (Phosphate

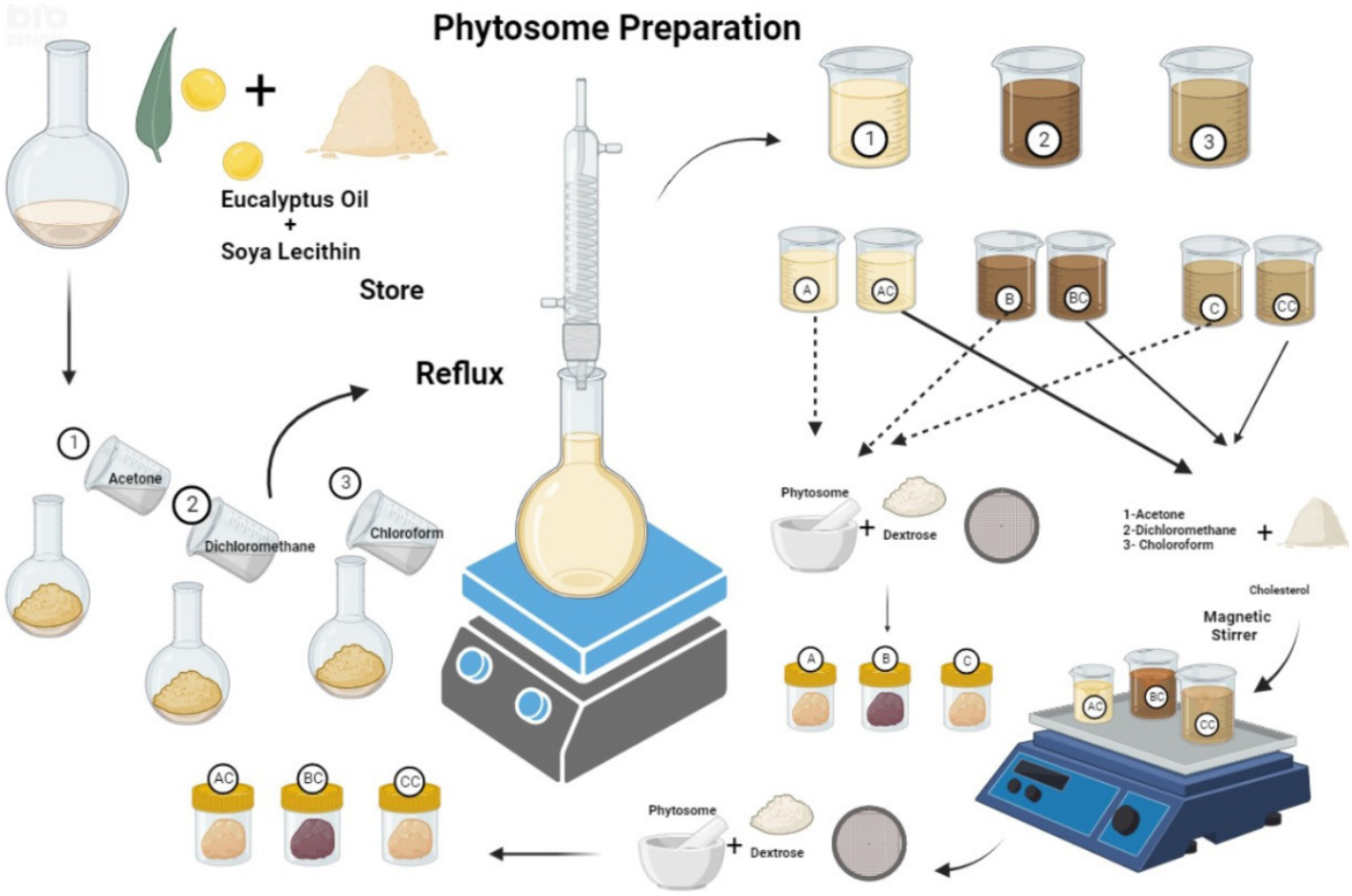


Figure 1: Preparation of phytosome.

Table 2: Composition of Eucalyptus oil phytosome.

Solvents	Acetone	Acetone + Cholesterol	Dichloromethane	Dichloromethane + Cholesterol	Chloroform	Chloroform + Cholesterol
Composition/ Code	A	AC	B	BC	C	CC
Solvent volume	50	50	50	50	50	50
[mL] Eucalyptus oil	5	5	5	5	5	5
Soya lecithin [mL]	2000	2000	2000	2000	2000	2000
Cholesterol[mg]	0	500	0	500	0	500
Dextrose [mg]	2000	2000	2000	2000	2000	2000

A, B, C: Without cholesterol & AC, BC, CC: With cholesterol

buffer pH 7.4) to maintain the sink condition. These samples were analysed at λ_{max} 297.733 nm by UV-spectrophotometer.[54]

Drug release kinetics

The drug release kinetics was determined by using BIT 1.12 software to confirm the type of drug release kinetics.[49]

Stability study

The optimized phytosome formulation was analyzed for stability study to study the physical appearance, leak out of the drug from phytosomes during storage. Optimized formulation was sealed in glass vials and stored at 4°C and room temperature and samples were regularly withdrawn at 7, 14, 21 and 28 days to examine the physical stability of prepared phytosomes.[55]

RESULTS AND DISCUSSION

Eucalyptus leaves ere authenticated by Mrs., Bnar Khalid Bakar at Salahaddin University Herbarium, Salahaddin University, Kurdistan as shown in Figure 2.

The eucalyptus oil was purified successfully by treatment with dichloromethane and using Rota vapor as shown in Figure 3.

Characterization of extracted oil Yield

The yield of oil was found to be 1.65±0.74%.

Density

The density was found to be 0.9928 g/cm^3 at 24.2°C as shown in Figure 4.

Refractive index

The refractive index was found to be 1.3613 at 24.6°C as shown in Figure 4.

UV-visible Spectrophotometry

The UV-visible spectroscopy showed the maximum absorbance (λ_{max}) 297.733 nm, and the line equation was found to be y = 0.008x-0.008 as shown in Figure 5.

FTIR of eucalyptus oil

The FTIR study showed the presence of (C-H) alkene stretching at 3000-2840 cm^{-1}, carbon dioxide (O=C=O) stretching at 2000-2400 cm^{-1}, alcohol (O-H) stretching, 3700-3584 cm^{-1}, (O-H) bending at 1420-1330 cm^{-1} (indicated weak group bending of aromatic compound), conjugated ketones (C=O) stretching at 1700-1690 cm^{-1}, primary amide functional group (C=O) at 1690 cm^{-1}. In the fingerprint region` alcohol (C-O) stretching as shown at 1050-1085 cm^{-1} as given in Figure 6.

Purification and drying of Soya lecithin E322 [Phospholipid]

The soya lecithin/ phospholipid were successfully purified and characterize by FTIR as shown in Figure 7.

Characterization of purified soya lecithin E322 (Phospholipid) by FTIR

The FTIR was used to confirm the groups and bond present in purified soya phospholipid showed in Figure 7. FTIR of impure soya lecithin and purified soya lecithin showed the presence of alcohol group at 3550-3200 cm^{-1}, broad O-H stretching at 3000-2840 cm^{-1}, C-H stretching of alkane and stretching of broad (N=C=O) at 2275-2250 cm^{-1}. The fingerprint region showed the presence of strong stretching of conjugated anhydride (C=O) at 1775 cm^{-1}, bending of methylene group at 1465 cm^{-1}, C-H, P-O-C at 1145-970 cm^{-1}, P-O-C + PO2 at 1200-970 cm^{-1}, PO2 at 1145-970 cm^{-1} as shown in Figure 7.

Drug-excipient interaction

The FTIR of dextrose showed the presence of (O-H) stretching strong at 3200–3350 cm^{-1}. The fingerprint region showed the stretching of secondary alcohol (C-O) at 1087-1124 cm^{-1}, bending of phenolic (O-H) at 1310-1390 cm^{-1}, stretching of primary alcohol (C-O) at 1050-1085 cm^{-1}, (C-H) bending for CO of carboxylic acid at 1720 cm^{-1} and 1200 cm^{-1}, C-C *Vib* sp^2 hybridization at 1600 cm^{-1} as shown in Figure 7.

Figure 2: Herbarium of Eucalyptus leaves and plant authentication

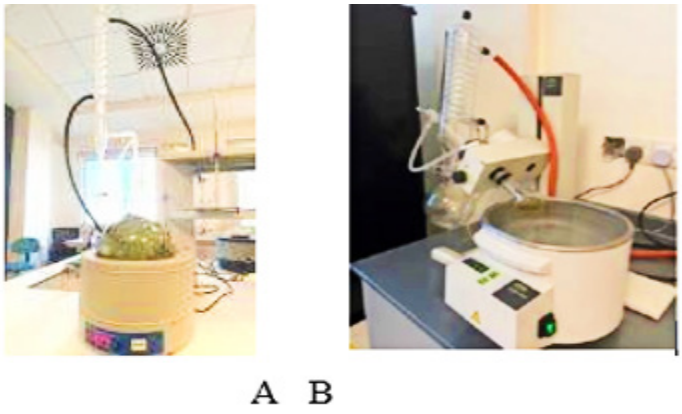


Figure 3: (A) Extraction of volatile oil, (B) Purification of extracted volatile oil.

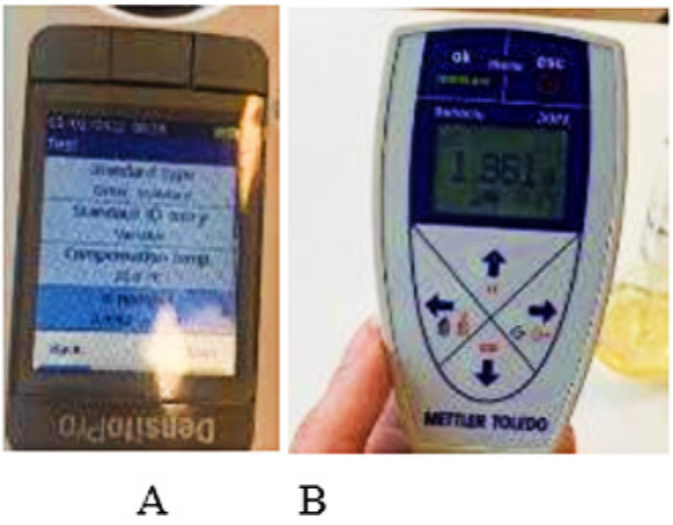


Figure 4: Eucalyptus oil (A) Density, (B) Refractive Index.

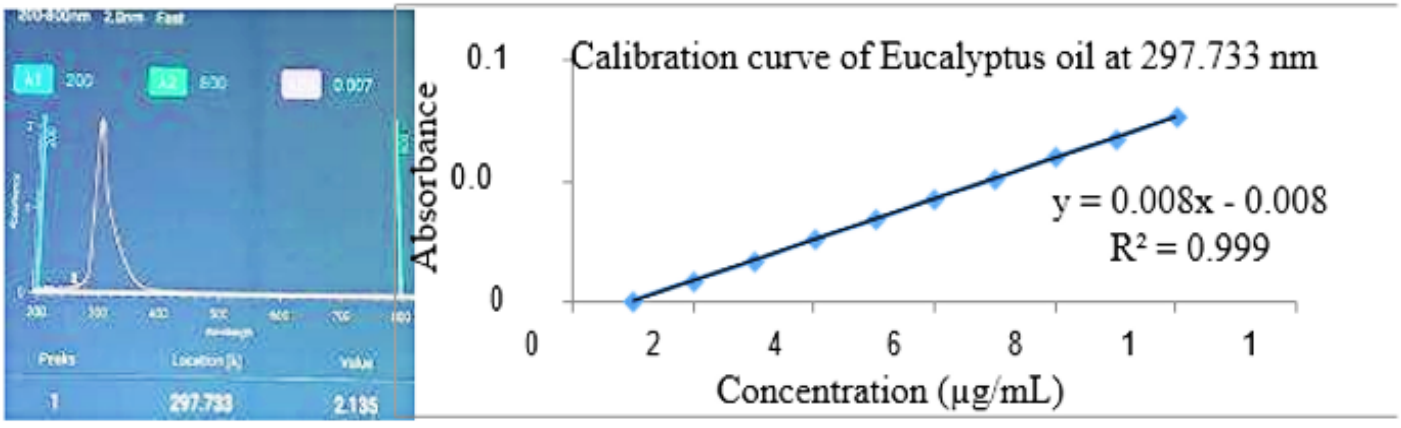


Figure 5: (A) Maximum absorbance (λ_{max}) of Eucalyptus oil, (B) Calibration curve of Eucalyptus oil at 297.733 nm.

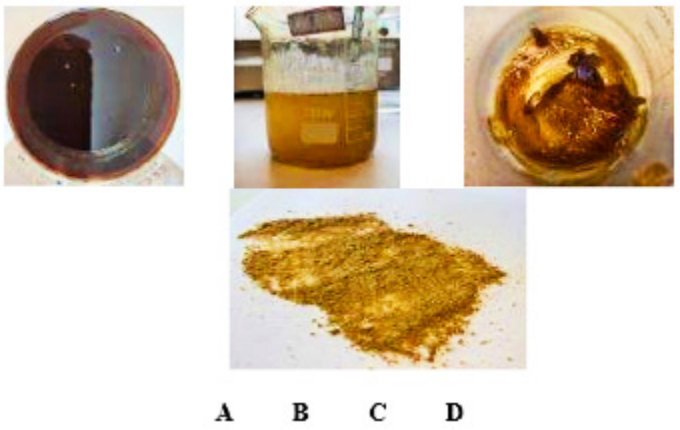


Figure 6: FTIR of (A) FTIR of eucalyptus oil, (B) FTIR of soya lecithin (Impure), (C) FTIR of soya lecithin (Purified), (D) FTIR of dextrose, (E) FTIR of cholesterol.

The FTIR of cholesterol showed the presence of (C-H) stretching of alkane at 2840–3000 cm^{-1}. The fingerprint region showed C-H bending around 1450-1500 cm^{-1}, C-O stretching of secondary alcohol at 1087-1124 cm^{-1}, phenol O-H bending at 1310-1390 cm^{-1}, C-H bending of alkane at 1087-1124 cm^{-1}, Asy. and Sy. CH_2 and CH_3 group at 2800-3000 cm^{-1}, OH group at 3400 cm^{-1}, CH2Sy. St. Vib. at 2899 cm^{-1}, C=C Sy. St. Vib. at 1677 cm^{-1}, cyclopentene at 1693-1671 cm^{-1}, Asy St. Vib. CH_2 and CH_3 at 1464 cm^{-1}, CH2 and CH3 Bend Vib at 1378 cm^{-1}, C-C-C St. Vib. at 840 cm^{-1}, CH Out of plan bend at 900-675 cm^{-1}, 700 cm^{-1}, 739 cm^{-1}, 800 cm^{-1}, 885 cm^{-1}) that indicated the presence of aromatic substance, CH Vib at 1689-1994 cm^{-1} overtone and reflect substitution pattern on ring, 927 cm^{-1}, 985 cm^{-1} as shown in Figure 7. The drug excipient interaction was not found as the FTIR of all phytosome batches as indicated in Figure 8, indicated the stretching of alkyne C-H at 3200–3350 cm^{-1}, C-H Asym./sym stretch at 2850–2815 cm^{-1}. In fingerprint region 1087-1124 cm^{-1} indicated the presence of secondary alcohol, C-O stretching at 1310-1390 cm^{-1}, P-O-C at 1145-970 cm^{-1}, P-O-C + PO_2 at 1200-970 cm^{-1}, PO2 at 1145-970 cm^{-1}. 1720 cm^{-1}, 1200 cm^{-1} for CO of carboxylic acid, 1600 cm^{-1} for C-C Vib sp2 hybridization as shown in Figure 8.

Cholesterol

Preparation of Eucalyptus phytosome by reflux method using different solvents

The six phytosome batches of eucalyptus oil were prepared successfully as shown in Figure 9.

Evaluation of phytosomes Yield

The yield of phytosome varied between 85.14±0.74 to 87.14±0.74% as shown in Table 3 and depicted in the Figure 10.

Particle size measurement

The particle size for all 6 batches was measured and size of phytosome varied from 71.76±0.63 to 197.36±0.53 nm as shown in Table 3 and depicted in the Figure 11 and Figure 12.

Zeta potential

The zeta potential of all six batches were measured and varied from 15.3±0.27 to-28.2±0.26 mV as shown in Table 3 and depicted in the Figure 13 and Figure 14.

Entrapment efficiency

The entrapment efficiency varied from 57.45±0.35 to 67.34±0.52 as shown in Table 3 and depicted in the Figure 15.

In vitro drug release

The *in vitro* drug release varied from 85.15±0.63 to 87.26±0.34% at 300 min. (5 hr) as data showed in Table 4 and depicted in Figure 16.

Batch "A" showed the maximum drug release 87.26±0.63% up to 5 hr. Peppas Korsmeyer was the best fit model with R^2 value 0.9422. The mechanism of drug release was Fickian Diffusion (Higuchi Matrix) as shown in Table 5 and Table 6.

Stability study

The stability studies showed 99.14±0.25 to 99.55±0.52% drug content on 28th day as showed in Table 7 and depicted in Figure 17.

DISCUSSION

Mostly non-polar aprotic solvents are best to prepare the phytosomes that provide proper interactions among phospholipids and polyphenols.[56] The size of the phytosome varied in following order; Acetone<Dichloromethane<Chlorof orm can be a reared to molecular weight, molecular diameter and dielectric constant of solvents that resulted in largest particle size of phytosome with chloroform while smallest particle size with acetone. The reaction/ rearrangement patter of phospholipid/ cholesterol molecules varied depending on an increase in molecular weight and molecular diameter and decrease in dielectric constant (increased non-polarity) of solvents. With acetone, the molecular weight and molecular diameter were decreased and dielectric constant (non-polarity) of solvents was increased resulted in formation of small particles of phytosome. With dichloromethane, the molecular weight and molecular diameter and dielectric constant (non-polarity) of solvents was in-between resulted in formation of medium size particles of phytosome. With chloroform, the molecular weight and molecular diameter were increased and dielectric constant (non-polarity) of solvents was decrease resulted in formation of large particles of phytosome. On addition of cholesterol the particle size of phytosome were increased as cholesterol also assembled/ orient in-between phytosome during phytosome formation/ construction. The zeta potential of phytosome was found to be decreased on addition of cholesterol. The phytosome prepared using acetone showed the smallest particle size and highest drug release while phytosome prepared using chloroform showed

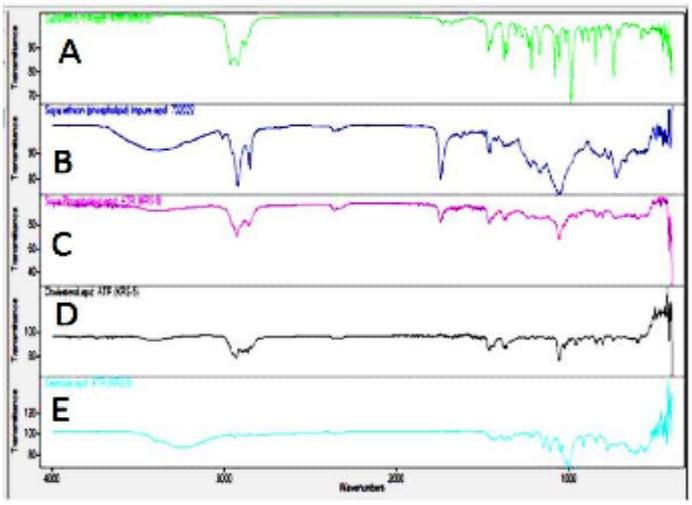


Figure 7: FTIR of (A) FTIR of eucalyptus oil, (B) FTIR of soya lecithin (Impure), (C) FTIR of soya lecithin (Purified), (D) FTIR of dextrose, (E) FTIR of cholesterol.

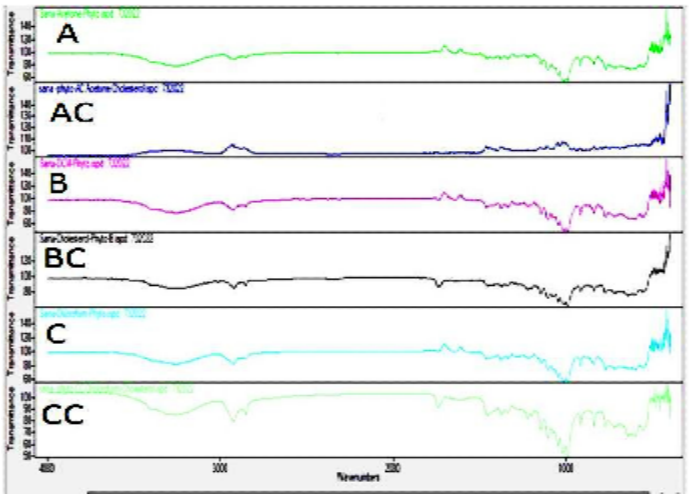


Figure 8: (A) FTIR of Batch A (acetone), AC (acetone + cholesterol), (B) (dichloromethane), BC (dichloromethane + cholesterol), (C) (Chloroform),CC (Chloroform+ cholesterol).

Figure 9: Preparation of Eucalyptus phytosome by reflux method using different solvents.

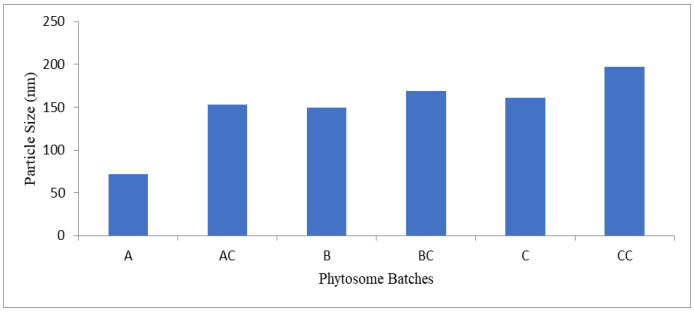


Figure 11: Particle size of phytosomes.

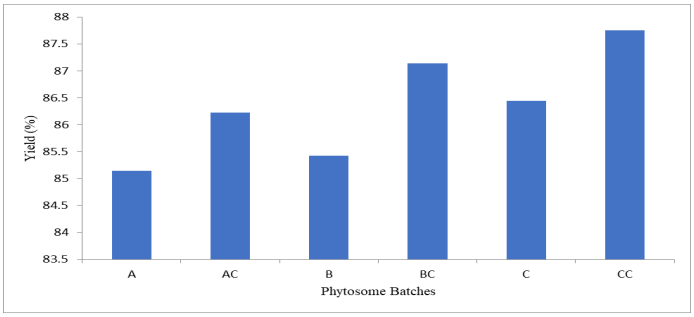


Figure 10: Yield of phytosomes.

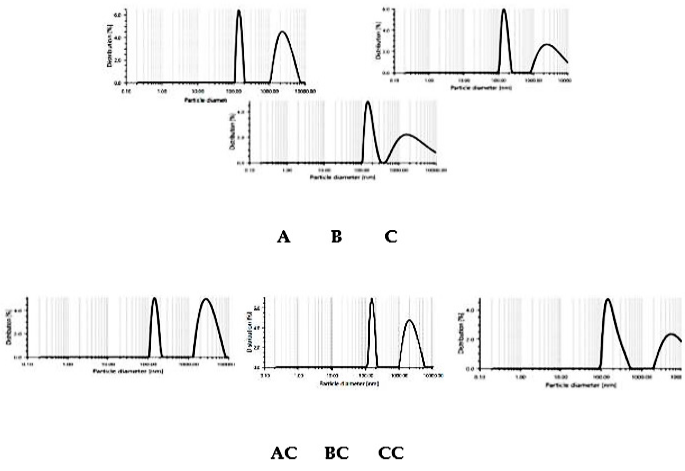


Figure 12: Particle size graph of phytosomes.

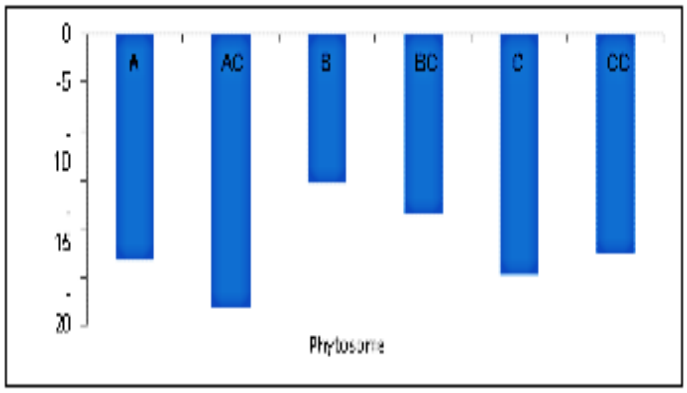


Figure 13: Zeta potential of phytosomes.

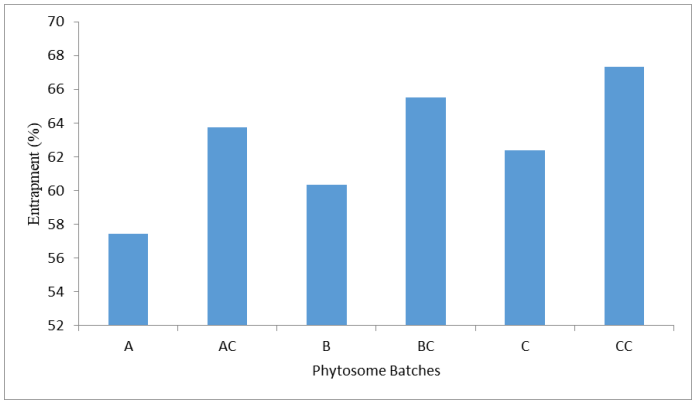


Figure 15: Entrapment of phytosomes.

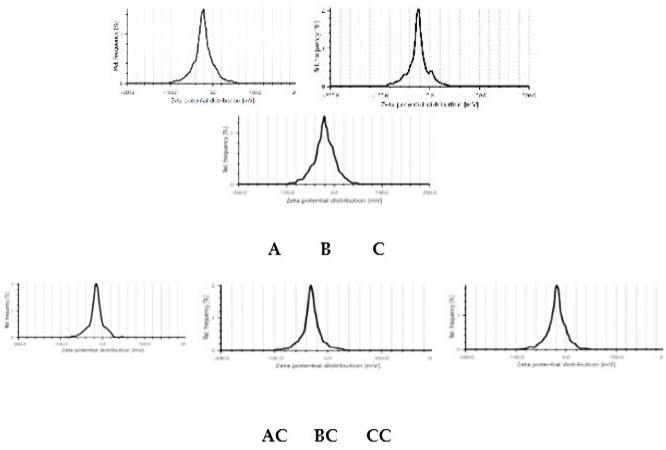


Figure 14: Zeta potential graph of phytosomes.

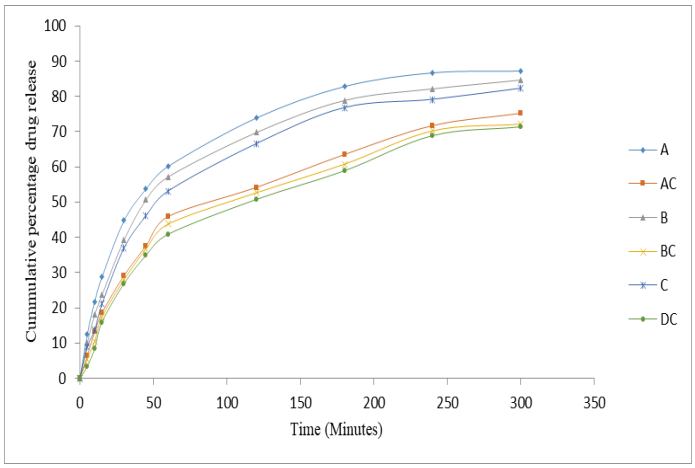


Figure 16: *In-vitro* drug release.

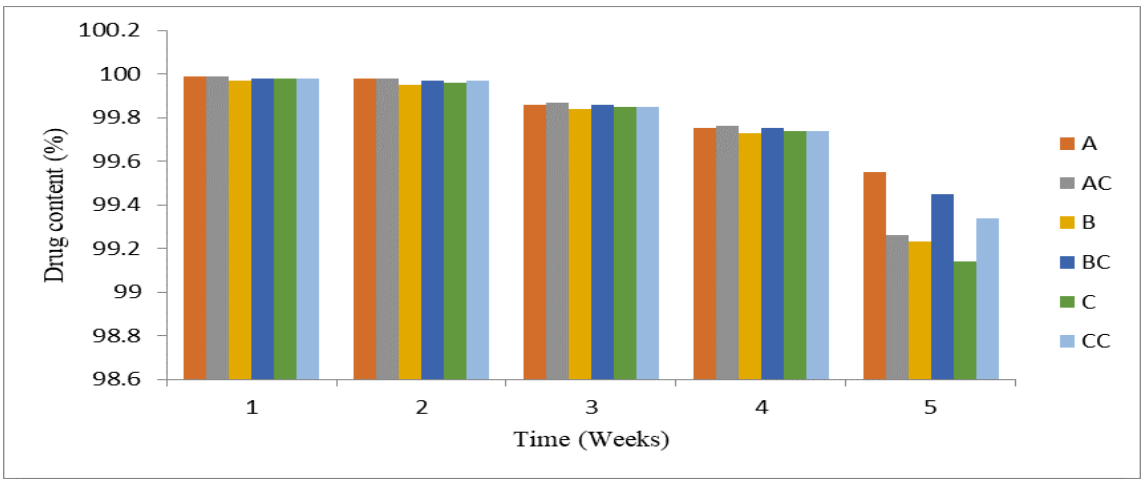


Figure 17: Drug content of phytosome in stability studies.

Table 3: Yield, particle size, zeta potential and entrapment of phytosomes.

Code	Yield (%)	Particle size (nm)	Zeta potential (mV)	Entrapment (%)
A	85.14±0.74	71.76±0.63	-23.3±0.34	57.45±0.35
AC	86.22±0.36	153.31±0.27	-28.2±0.26	63.74±0.63
B	85.42±0.26	149.73±0.84	-15.3±0.27	60.35±0.24
BC	87.14±0.74	168.73±0.37	-18.5±0.35	65.53±0.51
C	86.44±0.62	160.9±0.27	-22.6±0.36	62.37±0.62
CC	87.75±0.32	197.36±0.53	-24.7±0.12	67.34±0.52

Table 4: *In vitro* drug release from phytosomes.

Time (Min.)	A	AC	B	BC	C	DC
0	0	0	0	0	0	0
5	12.43±1.32	6.47±0.63	9.93±0.34	5.42±1.23	8.95±1.51	3.39±1.24
10	21.75±0.45	13.14±1.46	17.98±1.51	10.55±0.63	13.64±2.14	8.45±0.63
30	44.74±1.53	29.13±0.84	39.25±1.35	27.98±0.26	36.97±0.26	26.89±1.63
45	53.75±0.62	37.56±1.25	50.65±2.62	36.74±1.25	46.1±0.63	34.9±2.64
60	60.15±1.63	45.85±2.53	57.15±0.23	43.78±1.15	53.12±1.23	40.88±0.35
120	73.9±0.73	54.12±0.23	69.74±0.42	52.65±0.22	66.55±1.15	50.75±1.23
180	82.85±2.63	63.43±2.35	78.85±2.14	60.78±0.53	76.78±2.24	58.87±0.64
240	86.75±1.53	71.64±0.53	82.15±0.63	70.25±1.24	79.11±0.15	68.85±1.35
300	87.26±0.63	75.11±1.24	84.64±1.34	72.15±0.45	82.25±0.53	71.35±0.63

Table 5: Drug release kinetics by all batches.

Drug release kinetics						
Parameters	**A**	**B**	**C**	**AC**	**BC**	**DC**
Best fit model	Peppas Korsmeyer	Peppas Korsmeyer	Peppas Korsmeyer	Peppas Korsmeyer	Peppas Korsmeyer	Peppas Korsmeyer
R^2	0.9422	0.9422	0.9458	0.9518	0.9425	0.9501
Mechanism of release	Fickian Diffusion (Higuchi Matrix)	Anomalous Transport	Anomalous Transport	Anomalous Transport	First order	First order

Table 6: Drug release kinetics by batche-A.

Model Fitting	R^2	K
Zero order	0.7599	0.2594
1st order	0.9275	-0.007
Higuchi Matrix	0.9214	10.2379
Peppas	0.9422	2.4397
Hix.Crow.	0.8807	0.0016
Parameters for Korsmeyer-Peppas Equation	N 0.4594	
Best fit model Peppas Korsmeyer	Peppas Korsmeyer	
Mechanism of release	Fickian Diffusion (Higuchi Matrix)	

Table 7: Drug content of phytosome in stability studies.

Time (Weeks)	A	B	C	AC	BC	CC
0	99.99±0.83	99.97±0.23	99.98±0.16	99.99±0.37	99.98±0.44	99.98±0.36
7	99.98±0.79	99.95±0.22	99.96±0.13	99.98±0.34	99.97±0.45	99.97±0.33
14	99.86±0.74	99.84±0.19	99.85±0.21	99.87±0.98	99.86±0.84	99.85±0.98
21	99.75±0.63	99.73±0.97	99.74±0.89	99.76±0.87	99.75±0.78	99.74±0.96
28	99.55±0.52	99.23±0.72	99.14±0.25	99.26±0.16	99.45±0.36	99.34±0.25

the largest particle size and low drug release. The phytosome batch prepared using acetone and phospholipid showed more drug release than phytosome prepared with phospholipid and cholesterol combination that indicate that cholesterol improved the stability of phytosome as phytoconstituents can be entrapped or complex with phospholipid. The drug release was decreased on addition of cholesterol due to hydrophobic composition or assembly of cholesterol in phytosome as phytoconstituents entrapped in phospholipid and phytoconstituents complex with phospholipid both are considered as phytosome. The cholesterol increased the stability of phytosome due to their hydrophobic behavior and so the drug release was less with phytosome batch prepared with acetone with cholesterol rather than the phytosome batch prepared with acetone alone.

CONCLUSION

The particle size of phytosomes was increased on addition of cholesterol and also the solvent affects the particle size of phytosome as acetone showed the smallest particle size and chloroform showed the biggest particle size. It can be concluded that molecule diameter and molecular weight affects the solubility and assembly of phospholipid/ cholesterol in solvent in order to prepare particulate drug delivery system. The zeta potential was decreased on addition of cholesterol.

ACKNOWLEDGEMENT

We wish to thank Tishk International University to provide every necessary support for this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

ABBREVIATIONS

λ_{max}: Maximum absorbance; **%:** Percentage; **g:** gram; **cm^3:** Centimetre cube; **°C:** Degree centigrade; **nm:** Nanometre; **mV:** Millivolt; **NDDS:** Novel drug delivery systems; **TDDS:** Targeted drug delivery systems; **PC:** Phosphatidylcholine; **URI:** Upper respiratory infections; **RBF:** Round bottom flask; **mL:** Millilitre; **DCM:** Dichloromethane; **mg:** Milligram; **µg:** Microgram; **$CHCl_3$:** Chloroform; **FTIR:** Fourier transform inferred spectroscopy; **cm^{-1}:** Per centimetre; **rpm:** Revolution per minute; **UV:** Ultraviolet; **VIS:** Visible; **pH:** Potential of hydrogen; **Asy:** Asymmetric; **Sy:** Symmetric; **St:** Stretching; **Vib:** Vibrations.

SUMMARY

The volatile oil of eucalyptus was extracted by hydro-distilled using Clevenger apparatus and purified vacuum rotary evaporator (Rota vapor) and solvent Dichloromethane (DCM). The phytosome of eucalyptus oil were prepaid using reflux method with three different solvents (acetone, dichloromethane, and chloroform). These three batches were further divided into two groups i.e., with cholesterol and without cholesterol. The dextrose was further added as re-hydrant in all batches. The size of the phytosome varied in following order; Acetone<Dichloromethan e<Chloroform can be a regarded to molecular weight, molecular diameter and dielectric constant of solvents that resulted in largest particle size of phytosome with chloroform while smallest particle size with acetone. On addition of cholesterol the particle size of phytosome were increased as cholesterol also assembled/ orient in-between phytosome during phytosome formation/ construction. The zeta potential of phytosome was found to be decreased on addition of cholesterol. The cholesterol increased the stability ofphytosome. The particle size of phytosomes was increased on addition of cholesterol. It can be concluded that molecule diameter and molecular weight affects the solubility and assembly of phospholipid/ cholesterol in solvent in order to prepare particulate drug delivery system. The zeta potential was decreased on addition of cholesterol.

REFERENCES

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Mol. 2016;21(5):559-77.
2. Pananchery J, Gadgoli C. Phytosomes of naphthoquinone enriched extract of root bark of onosma echioides exhibit wound healing activity in rats. Indonesian J. Pharm. 2021:474-83.
3. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, *et al*. Important flavonoids and their role as a therapeutic agent. Mol. 2020;25(22):5243-82.
4. Aqil F, Munagala R, Jeyabalan J, Vadhanam MV. Bioavailability of phytochemicals and its enhancement by drug delivery systems. Cancer Lett. 2013;334(1):133-41.
5. Pahwa R, Chetna, Saroha K, Bhardwaj B, Kumar M, Singh I. Insights into various approaches of phytosomes for enhanced therapeutic potential of bioactives. Res J Pharm Technol. 2022;15(9):4277-82.
6. Kesarwani K, Gupta RK, Mukerjee A. Bioavailability enhancers of herbal origin: An overview. Asian Pac. J. Trop. Biomed. 2013;3(4):253-66.
7. Barani M, Sangiovanni E, Angarano M, *et al.* Phytosomes as innovative delivery systems for phytochemicals: a comprehensive review of literature. Int J Nanomedicine. 2021;16:6983-7022.
8. Kidd P and Head K. A Review of the Bioavailability and Clinical Efficacy of Milk Thistle Phytosome: A Silybinphosphatidylcholine Complex. Altern Med Rev. 2005;10:193-203.
9. G S R, Rompicherla NC, Dubey A, Hebbar S, Mathias A. Phytosomes: A Novel Molecular Nano Complex Between Phytomolecule and Phospholipid as a Value added Herbal Drug Delivery System. International Journal of Pharmaceutical Sciences Review and Research. 2018;51:84-90.
10. Singh A, Saharan VA, Singh M, Anil B. Phytosome: Drug delivery system for polyphenolic phytoconstituents. Iran. J. Pharm. Sci. 2011;7(4-7):209-219.
11. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, *et al*. A review on phospholipids and their main applications in drug delivery systems. Asian J. Pharm. Sci. 2014;10(2),81-98.
12. Drescher S, van Hoogevest P. The phospholipid research center: current research in phospholipids and their use in drug delivery. Pharmaceutics. 2020;12(12):1235-1271.
13. Pichot R, Watson RL, Norton IT. Phospholipids at the interface: Current trends and challenges. Int J Mol Sci. 2013;14(6):11767-11794.
14. Nagle JF, Tristram-Nagle S. Lipid bilayer structure. Curr Opin Struct Biol. 2000;10(4):474-480.
15. Meineke J, Weik M, Zaccai G, Fragneto G. Behavior of hydrated lipid bilayers at cryogenic temperatures. Front Chem.2020; 8:455-462.
16. Edidin, M. Lipids on the frontier: a century of cell-membrane lipids Nature Reviews: Molecular Cell Biology. 2003;4:414–418.
17. Kanno K, Wu MK, Scapa EF, Roderick SL, Cohen DE. Structure and function of phosphatidylcholine transfer protein [PC-TP]/StarD2. Biochim Biophys Acta. 2007;1771(6):654-662.
18. Alharbi WS, Almughem FA, Almehmady AM, Jarallah SJ, Alsharif WK, Alzahrani N, *et al*. Phytosomes as an emerging nanotechnology platform for the topical delivery of bioactive phytochemicals. Pharmaceutics. 2021; 13:1475-95.
19. Singh A, Singh A. Phyto-Phospholipid Complexes: A potential novel carrier system for improving bioavailability of phytoconstituents. Res J Pharm Technol. 2020;13(2):1059-66.
20. Bhattacharya S. Phytosomes: The new technology for enhancement of bioavailability of botanicals and nutraceuticals. Int. J. Health Res. 2009;2(3):225-32.
21. Nagar G. Phytosomes: A novel drug delivery for herbal extracts. Int J Pharm Sci Res.2019; 9(3-s):924-30.
22. Kashapov R, Gaynanova G, Gabdrakhmanov D, Kuznetsov D, Pavlov R, Petrov K, *et al*. Self-assembly of amphiphilic compounds as a versatile tool for construction of nanoscale drug carriers. Int. J. Mol. Sci. 2020;21(18):6961-7008.
23. Grgić J, Šelo G, Planinić M, Tišma M, Bucić-Kojić A. Role of the encapsulation in bioavailability of phenolic compounds. Antioxidants. 2020;9(10):923-58.
24. Lu M, Qiu Q, Luo X, Liu X, Sun J, Wang C, *et al*. Phyto-phospholipid complexes [phytosomes]: A novel strategy to improve the bioavailability of active constituents. Asian J. Pharm. Sci. 2019;14(3):265-74.
25. Shriram RG, Moin A, Alotaibi HF, Khafagy E-S, Al Saqr A, Abu Lila AS, *et al*. Phytosomes as a plausible nano-delivery system for enhanced oral bioavailability and improved hepatoprotective activity of silymarin. Pharmaceuticals. 2022;15(7):790-810.
26. Chen R-P, Chavda VP, Patel AB, Chen Z-S. Phytochemical delivery through transferosome (phytosome): an advanced transdermal drug delivery for complementary medicines. Front. Pharmacol.2022;13:850-862.
27. Nakhaei P, Margiana R, Bokov DO, Abdel basset WK, Jadidi Kouhbanani MA, Varma RS, *et al*. Liposomes: Structure, biomedical applications, and stability parameters with emphasis on cholesterol. Front. Bioeng. Biotechnol. 2021;9(9):705886.
28. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, *et al*. Liposome: Classification, preparation, and applications. Nanoscale Res Lett. 2013;8(1):102-9.
29. Bharati R, Badola A. Phytosomes–A modernised and new technology: Revolutionary progress in the field of pharmacy for enhanced bioavailability of cosmeceuticals and neutraceuticals. World J. Pharm. Res. 2021;10(10): 186-202.
30. Bijak M. Silaybin, A major bioactive component of milk thistle [*Silybum marianum* L. Gaernt.]-chemistray, bioavailability, and metabolism. Molecules. 2017;22(11):1942-1953.
31. Aziz ZAA, Nasir HM, Ahmad A, Setapar SHM, Ahmad H, Noor MHM, *et al*. Enrichment of eucalyptus oil nanoemulsion by micellar nanotechnology: transdermal analgesic activity using hot plate test in rats' assay. Sci. Rep. 2019;9(1): 13678.
32. Sebei K, Sakouhi F, Herchi W, Khouja ML, Boukhchina S. Chemical composition and antibacterial activities of seven eucalyptus species essential oils leaves. Biol. Res. 2015;48(1):1-5.
33. Sugumar S, Clarke S, Nirmala M, Tyagi B, Mukherjee A, Chandrasekaran N. Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. Bull. Entomol. Res. 2014;104(3):393-402.
34. Madreseh-Ghahfarokhi S, Dehghani-Samani A, Pirali Y, Dehghani-Samani A. *Zingiber officinalis* and eucalyptus globulus, potent lethal/repellent agents against Rhipicephalus bursa, probable carrier for zoonosis. J. Arthropod. Borne Dis. 2019;13(2):214-23.
35. Sheikh Z, Amani A, Basseri HR, Kazemi SHM, Sedaghat MM, Azam K, *et al*. Repellent efficacy of eucalyptus globulus and Syzygium Aromaticum essential oils against malaria vector, Anopheles stephensi [Diptera: Culicidae]. Iran. J. Public Health. 2021;50(8):1668.
36. Maciel M, Morais S, Bevilaqua C, Silva R, Barros R, Sousa R, *et al*. Chemical composition of eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. Vet. Parasitol. 2010;167(1):1-7.
37. Halim ASA, Morsy TA.The insecticidal activity of eucalyptus globulus oil on the development of Musca domestica third stage larvae. J Egypt Soc Parasitol. 2005 Aug;35(2):631-6.
38. Barbosa LCM, Filomeno CA, Teixeira RR. Chemical variability and biological activities of eucalyptus spp. Essential Oils. Molecules.2016; 21:1671-704.
39. Ali Esmail Al-Snafi. Traditional uses of Iraqi medicinal plants. IOSR J. Pharm.2018;8(8-II):32-95.
40. Izham, M.N.M., Hussin, Y., Rahim, N.F.C. *et al*. Physicochemical characterization, cytotoxic effect and toxicity evaluation of nanostructured lipid carrier loaded with eucalyptol. BMC Complement Med Ther. 2021;21:254.
41. Horváth G, Ács K. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: A review. Flavour Fragr. J. 2015;30(5):331-41.
42. Visht S, Rathi V. Antibacterial activity of volatile oil from areal plant. Int. J. Curr. Adv. Res.2018; 07(4):11446-8.
43. Gao X, Lv S, Wu Y, Li J, Zhang W, Meng W, *et al*. Volatile components of essential oils extracted from Pu-erh ripe tea by different extraction methods. Int. J. Food Prop. 2017;20(1):S240-S253.
44. Yuan Y, Huang M, Pang Y-X, Yu F-L, Chen C, Liu L-W, *et al*. Variations in essential oil yield, composition, and antioxidant activity of different plant organs from Blumea balsamifera [L.] DC. at different growth times. Molecules. 2016;21(8):1024.
45. Rombaut N, Savoire R, Thomasset B, Castello J, Van Hecke E, Lanoisellé J-L. Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. Ind Crops Prod.2015; 63:26-33.

46. Visht S, T Kulkarni G. Glycyrrhetinic acid ammonium loaded microspheres using colocasia esculenta and bombax ceiba mucilages: In-vitro and in-vivo characterization. Curr. Drug ther. 2016;11(2):101-14.
47. Visht S, Anjum N, Saini A. Comparison of drug release: Microparticles vs nanoparticles. Int. Res. J. Pharm.2018; 9:52-58.
48. Ooki Takamasa; Sato Munetaka; Kudo Satoshi (1988). Lecithin purification method (JPH01197492A) [Publication JP2503567B2], Title:Method for refining lecithin, Publication date 1996-06-05], [Publication JPH01197492A, Title Method for refining lecithin, Publication date 1989-08-09, Applicants Japan Res and Dev Ass, Current Assignee Niigata EngineeringCoLtd] [https://patents.google.com/patent/JP2503567B2/en] [https://worldwide.espacenet.com/patent/search/family/012101884/publication/JP2503567B2?q=pn%3DJP2503567B2]
49. Visht S, Kulkarni G. Studies on the Preparation and *in vitro-in vivo* evaluation of mucoadhesive microspheres of glycyrrhetinic acid isolated from liquorice. Bangladesh J. Pharmacol. 2015; 18:30-7.
50. Mummidi V and Kondamuri P: Phytosomes: A new vesicular delivery for herbal medicine. Int J Pharm Sci and Res. 2021;12(11):5634-41.
51. Fahmy UA, Ahmed OA, Hosny KM. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS Pharm Sci Tech. 2015;16(1):53-8.
52. Shah B, S. B. Puranik, Raghuchandan H S, Preparation and evaluation of norbixin phytosomes, Int. j. sci. res. methodol. 2020;15(1):238-63.
53. Kumar A, Kumar B, Singh SK, Kaur B, Singh S. A review on phytosomes: novel approach for herbal phytochemicals. Asian J Pharm Clin Res. 2017;10(10):41-7.
54. Uhljar LÉ, Kan SY, Radacsi N, Koutsos V, Szabó-Révész P, Ambrus R. *In vitro* Drug Nanofibers. Pharmaceutics. 2021;13(4):556.
55. Visht S, Awasthi R, Rai RS, Srivastav P. Development of dehydration-rehydration liposomal system using film hydration technique followed by sonication. Curr. Drug Deliv. 2014;11(6):763-70.
56. Shakeri A, Sahebkar A. Phytosome: A Fatty Solution for Efficient Formulation of Phytopharmaceuticals. Recent Patents on Drug Delivery and Formulation, 2016;10:1-7.

Cite this article: Visht S, Salih SS. Effect of Cholesterol and Different Solvents on Particle Size, Zeta Potential and Drug Release of Eucalyptus Oil Phytosome. Pharmacog Res. 2023;15(3):578-90.