Plant Archives Vol. 21, Supplement 1, 2021 pp. 2674-2682 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.436

ISOLATION AND PHYTOCHEMICAL SCREENING OF *CITRULLUS COLOCYNTHIS* FORMULATION

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ABSTRACT

Objective: Present work is aimed at preparation and characterization of phytosomes, one of the, novel drug delivery dosage form used for improving bioavailability of drug, formed from ethanolic, aqueous, dichloromethane and ethyl acetate extract of *Citrullus colocynthis* fruit. Method: Required phytosomal formulations were developed in the form of different extracts of *Citrullus colocynthis* fruit from the lecithin and cholesterol, Then characterization involved various parameters, such as: Particle size determination, apparent solubility, entrapment efficiency (EE%), scanning electron microscope (SEM), xrd, FTIR spectroscopy and dissolution study. Result and Conclusion: Particle size varied from 250nm to 0.1µm of different formulations. The mean particle size of phytosome of aqueous extract of CC was scattered in a narrow range of 254±20.0 nm, and polydispersity index was 0.857±0.055, where phytosome of aqueous extract shows better entrapment then other phytosomal formulation. Photomicrographs showed that phytosomes were circular in shape and consistent in size.

Keywords **:** *Citrullus colocynthis*; phytosome; scanning electron microscope; fourier-transform infrared spectroscopy, x-ray diffraction (xrd), TLC and Entrapment efficiency.

Introduction

Ayurveda, the science, which provides a system of medical treatment that, is well advanced from hundreds of years. Many of the remedies, from plant as well as animal origin, were using for treating illness since ancient era. However, there is limited valuable information about Ayurveda is hardly available, because of the loss of various original Sanskrit treatises (Patwardhan *et al*., 2014). Though a part of the "Charaka Samhita", the oldest known medical treatise gives an vision of the qualities and effectiveness of crude\raw drugs, while another old treatise 'Sushruta Samhita' covers an comprehensive description of about 700 plants, grouped into 37 classes (Pal, Shukla, 2003).

With the rapid development and improvement in various fields of human activities, the fields of medicine and allied sciences have also made rapid paced. The synthesis of many therapeutic drugs has certainly modernized the treatment of diseases. Now a day, large numbers of synthetic drugs are available to fight with different unwell health conditions. It is a fact that these drugs are not truly away from adverse effects (Verma, Singh, 2008). They are known to be responsible for proliferation of side reactions and estrogenic diseases. Another concern for belief about indigenous practices may be due to the price. Most of the medicines are unusually costly beyond reach of ordinary man. That is why; researchers appreciate their purpose to be in the field of indigenous, where the drugs obtained from our land (Sharma *et al.*, 2008). Today, plants persevere their chronological significance as novel compounds either directly as medicinal remedial agents or as leading moiety for synthetic/semi-synthetic structural modifications or pharmacological studies.

It is noticeable that presently World Health Organization (WHO) is also encouraging traditional herbal plants based medicaments in natural health care programs in view of the fact that these drugs are economically readily feasible and are relatively not likely to harm (Ekor, 2014). The reappearance of plant-based drug is mainly based upon the growing evidence of the health hazards coupled with the harmful adverse effects of many synthetic medicines and random use of current system of medicine, which includes analgesics, steroids, antibiotics and various other synthetic drugs (Yuan *et al*., 2016). Now the plant and land based drugs are considered as fast growing industry.

Cucurbitaceae family is primarily distributed around the tropics (Rahmatullah *et al*., 2012). *Citrullus colocynthis* (L. Schrad.) indigenously found in humid climate of Asia and some regions of Africa (Ayyad *et al*., 2012). Generally, *C. colocynthis* plants are grow wild in the sandy lands and semi dry lands, is a perennial herb, they can extend their root system into deep groundwater and can stay alive in barren lands by sustaining their requirement of water without any sagging or dehydration (Ponsankar *et al*., 2018).

The phytosome technique has showed as the leading technique for enhancing bioavailability of phyto-pharmaceuticals having poor ability of solubilising and passing the biological membranes. Phytosome is a patented technology of Indena, where phospholipids are composited with plant-polyphenol to enhance their bioavailability (Lu *et al*., 2019), since phospholipids are too known as lipid

molecules and also glycerol is bonded to two fatty acids, such as, phosphatidylcholine, i.e., well known lipophilic moieties which readily can form complex with polyphenolic compounds. Phosphatidylcholine is a principal structural component of all biological membranes (Talware, Dias, Gupta, 2018).

The present study is aimed to develop extracts of CC loaded phospholipid complex that should be capable to enhance the bioavailability of the herbal drug. The composite thus prepared was evaluated physicochemically for crystallinity (X-RD), chemical interaction (FT-IR), surface morphology (SEM), solubility and dissolution rate study and spectroscopically. It is assumed that the developed phytosomes might be suitable for the reducing the amount of dose given and its frequency, hence reduce toxic or side effect of herbal drug (Hossain *et al*., 2011).

Materials and Methods

Collection and Authentication of plant material

The fruit of *C.colocynthis* had been collected from the area of Punjab and Rajasthan in the month, July 2016. The fruit part was identified in Raw Material Herbarium & Museum, NISCAIR, New Delhi, by Dr. Sunita Garg, under a specimen no. (NISCAIR/RHMD/Consult/-2017/3097-46), that has been deposited at the herbarium unit.

Morphological studies

The morphological/organoleptic evaluations were performed for selected plant material i.e. *Citrullus colocthnthis* fruit. The various organoleptic characters like, size, shape, texture, colour, odour and taste were evaluated for the fruit and its powder. The effect of addition of a small quantity of water to the powder, the effect of shaking the test tube containing powder with water, and the effect of pressing the little powder between the filter paper.

Preparation of plant extracts

The fruit of *C.colocynthis* was shade dried and powdered. 100 g of powder was then kept separately in 300 ml Dichloromethane (DCM) for maceration for three days with intermittent shaking, then filtered. The filtrate was further treated two times by using the same fresh solvent and all the filtrates were combined together. The filtrate so obtained was air dried and further extracted with ethyl acetate and followed by ethanol and aqueous medium similar to the process carried out for the DCM extraction. Finally, from each filtrate the solvent was evaporated using rotary evaporator under reduced pressure and low temperature. The yield of each extract was found and kept at 4°C until used. This was kept in airtight bottles in a refrigerator until used (Jeyaseelan *et al*., 2012).

Preliminary Phytochemical Screening

The different chemical tests were performed for various chemical constituents, as given in Table I (Kokate, Purohit, Gokhale, 2008).

Quantitative Screening of *Citrullus colocynthis*

Estimation of total flavonoid content (TFC):

TFC in different crude extracts were predicted with the help of aluminium chloride colorimetric method. Crude extract was reacted with 5% sodium nitrate solution and 10 % $AlCl_3$. Absorbance from UV spectrometer was taken after the addition of 4% NaOH at the wavelength of 420 nm (reported in literature). Quercetin had been used as standard for calibration curve. TLC was calculated by using the following formula:

$$X = \frac{A.mo}{Ao.m}$$

Where

"X" = Flavonoid content, (mg/g) plant extract,

"A" = Absorption of plant crude extract solution,

"Ao" = Absorption of standard quercetin solution,

"m" = Weight of crude drug extract, mg,

"mo" = Weight of quercetin in the solution, mg.

Thin Layer Chromatography:

Thin Layer Chromatography (TLC) is the most suitable method to detect the phytoconstituents (Marston *et al.,* 1997).

- Sample concentration: 20 mg/ml in Methanol (Test Extracts)
- Application volume: 5 µl
- Stationary phase: Silicagel 60 F_{254}
- Solvent system
- *Citrullus colocynthis* Chloroform: Methanol (90:10)
- Saturated chamber for 30 minute and approx run distance 8 cm
- Saturated chamber (30 min), aprox. run distant 8 cm• Detection Methods :
- Separated spots were visualized by visible, UV or iodine vapors.

Column Chromatography

The active extracts were separated on a silica gel column (Merck 70-230 mesh, 400 g, 3.5 i.d. ×150 cm) and consecutively eluted stepwise with gradient of Hexane, chloroform and methanol. After collection, each fraction was spotted on a pre-coated Silica gel 60 F_{254}, 0.25 mm thick TLC plate (Merck) with Iodine vapour and anisaldehyde spray reagent for identification of single compound elution.

Pre-coated Silica gel 60 F_{254}, 0.25 mm thick was spotted with total collected fractions. TLC plate (Merck) and eluted in hexane: ethyl acetate (3:1). Fractions with similar Rf values in TLC pattern were pooled together to get 5 fractions. The active fraction was rechromatographed on a silica gel column and eluted with a stepwise gradient of Hexane, chloroform and methanol solvent system. The obtained fractions were again eluted in 100% hexane elution then in last the active fractions with similar Rf values in hexane: chloroform 50:50 were pooled and confirmed as a pure compound. The compound was subjected to spectral analyses for structural determination.

Infrared Spectroscopy (IR)

IR spectra used to know the functional group present in compound, with the help of different vibrational energy levels.

Preparation and Characterisation of Phytosomes

Preparation of Phytosomes

Accurately weighed quantity of lecithin and cholesterol were dissolved in a mixture of 5 ml of chloroform and 5ml of acetone in round bottom flask (RBF). Thin layer of phospholipids mixture was formed by complete organic solvent removal in rotatory evaporator. This film was

hydrated with methanolic extract of fruit of *C.colocynthis* in rotary evaporator (37-40°C for 1 hour). Then the buffer (7.4 pH) was poured in the mixture of extract and phospholipids and rotated for 1 hr in rotary evaporator. Phytosomes were prepared. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8°C) until used.

Process variables used for optimization

- The developed formulation was optimized by selecting following process variables, as explained in Table II , Table III, Table IV and Table V.
- Effect of different extracts of the drug (Figure 1,2,3 and 4)
- Effect of lecithin concentration in preparation of phytosomes (Figure 5)

Characterization of Phytosomes

Apparent Solubility:

Add excess of CC extract or prepared phytosomes in 5ml of water or n-octanol at room temperature. Mixer was agitate for 1 day then centrifuged for 20 min to remove extra amount of CC extract or phytosome. Supernatant was filtered. Then absorbance was recorded at 268 nm using UV spectrophotometer.

Entrapment Efficiency:

Entrapment efficiency (EE) was calculated by using UV– visible spectrophotometer (UV-1601, Shimadzu). Measured quantity of phyto-phospholipid complex equivalent to CC extract with methanol and water in 1:2 stirred for 4 hr then centrifused for 15 min. Supernatant was filtered and absorbance was measured in UV at 268 nm; to measure the concentration of compound (Figure 1,2,3 and 4).

The EE (%) was estimated by using the given formula:

$$EE\ (\%) = T\text{-}S/T \times 100$$

Where, T-Total concentrations of CC extract,

S-is the CC extract contained in the filtrate.

Particle size distribution

Zetasizer was used for particle size determination, by stirring the mixer of isopropyl alcohol and compound for 10 min.

X-Ray diffraction (XRD) study

Diffractometer (Bruker, Germany) was used for the evaluation of the measurements of the samples. The operating conditions were current 0.8 mA; voltage 45 kV; scanning speed 1/min. The results were confirmed over a range of 5–60° (2θ) using the Cu-Anode X-ray tube and scintillation detector.

Fourier Transform Infrared spectroscopy (FTIR) Study

FT-IR studies were executed on pure CC, Cholesterol, and dichloromethane extract of CC, ethanolic extract of CC, ethyl acetate extract of CC and aqueous extract of CC was in an Alpha FTIR spectrophotometer IR Affinity^{-1} (Shimadzu Corporation).

Scanning electron microscopy (SEM)

Phytosomal formulations were viewed and photographed under scanning electron microscope.

Dissolution Study (in-vitro Drug Release)

The *in vitro* dissolution studies were performed in dissolution test apparatus. An exactly weighed quantity of phytosome of CC extract (50 mg) was with the phosphate buffer (pH 6.8). Then absorbance was measured in UV at 268 nm.

Results and Discussion

Phytochemical Evaluation of *Citrullus colocynthis:*

Morphological studies:

At the initial stage of the research work, the morphological assessment of Citrullus Colocynthis fruit was performed and the results obtained are mentioned in Table VI.

Preliminary Phytochemical analysis:

The preliminary Phyto-chemical analysis of all test extracts was performed (Kokate *et al*., 2008) and it is noted that aqueous extract of *Citrullus colocynthis* contains alkaloids, carbohydrates, tannins, steroids, and glycosides as shown in Table VII.

Quantitative Phytochemical Screening:

TFC was found high in the aqueous and ethyl acetate extracts of C. colocynthis fruits (48.32±0.13 and 46.34±0.03 mg QE/g), chased by ethanol extract and dichloromethane extract (59.84±0.90 and 28.7±1.04 mg QE/g), shown in Table VIII.

Thin layer chromatography:

In herbal Medicines, there are always hundreds of components and many of them are in too low amounts. On the other hand, the variability has been observed within the different and even the same herbal materials collected at different location or season. The TLC was performed for all extracts and the results obtained are mentioned in Table IX. The calculated RF values are also mentioned in below table and it was noted that the herbal extract contains number of chemical compounds like phenols, tannins, glycosides, vitamins, minerals it would difficult to conclude TLC results. So while deciding the quality control and standardization parameters all the assessment like morphology, macroscopic, microscopy, physicochemical and phytochemical parameter, elemental analysis has to be taken in to consideration.

Physico-chemical characterization of prepared phytosomes

Process Variable used for optimization: Explained in Table X and Table XI.

Apparent Solubility

Calculated apparent solubility of the CC extract and prepared phytosomes are shown in Table XII. CC extract had showed poor aq. solubility (4 µg/mL), and relatively prominent solubility in n-Octanol (305 µg/mL), representing a little lipophilic nature of the drug. The physical mixture (PM), i.e., phytosome composite depicted a non-significant variation in the n-Octanol solubility and a modest raise (1.5 times) in aqueous solubility. The prepared phytosome, although, demonstrated a notable, and a significant (over 12-fold) rise in the aqueous solubility. This elevate in the solubility of the prepared aqueous extract composite might be clarified by the partial amorphization, i.e., reduced molecular crystalline of the drug and the generally the amphiphilic character of the phytosome.

Particle size distribution

Surface area/volume (SA/V) ratio of the various particles is inversely proportional to their particle size. Therefore, we conclude that the smaller size of prepared phytosomes, encompassed a higher SA/V, that make it easier to entrap the drug that is released out from the phytosome via distribution and surface erosion. Additional benefit for the

drug-entrapped phytosomes, they are saturate through the physiological drug barriers. Previous studies showed that the small size particles (≤500 nm) might cross the cell membrane via endocytosis, while the large size particles (≤5 mm) are taken up via the lymphatics. The mean particle size of phytosomal complex CEA9 was scattered in a narrow range of 254±20.0 nm, and polydispersity index was 0.857±0.055.

X-Ray diffraction (XRD) study

The x-ray diffraction (XRD) patterns of phytosomes of CA7(1), CEA9(2), CE8(3) and CD6(4). The diffractogram of the 1, 2, 3 and 4 (shown in figure 6) revealed sharp crystalline peaks at 2θ=40.5°, 28° and 2.8°, 2θ=26°, 22.5° and 16°, 2θ=26°, 23° and 16° and 2θ=46.5°, 31° and 24° respectively.

Scanning electron microscopy (SEM)

SEM photographs shows the solid-state properties and surface morphology of extract of CC- phospholipid complex. In the figure 7, the crystalline state of Citrullus colocynth was picturised in the SEM photograph as many crystals. In figure drug was completely converted in to phyto-phospholipid complex where Citrullus Colocynth extract was physically wrapped by phytosome conveying amorphous nature of the complex due to which drug crystals was disappeared.

FT-IR study

Fourier transform infrared spectroscopy (FTIR) analysis of pure CC, Cholestrol, CA7, CE8, CD6 and CEA9 were studied in order to get insight into occurrence of interaction between extracts of CC and phospholipids. The FTIR spectrum of CC displayed a broad peak at 3440 cm^{-1}, the aliphatic alcoholic (–OH) group, 1637 cm^{-1} (Conjugated ester stretching), 1414- 1558 cm^{-1} (aromatic signals), 1400-1600 cm^{-1} (Aromatic absorptions stretching). FTIR spectrum of Cholestrol revealed the characteristic absorption at 2800-3000 cm^{-1} (-CH_2 and -CH_3 groups), 3442 cm^{-1} (-OH stretching), 1639 cm^{-1} (-C=O group), 1375 cm^{-1} (-$CH_2$and -CH_3 bending vibration), 1055 cm^{-1} (ring deformation). The FTIR spectrum of the prepared phytosomes from the different extracts of CC is quite different from that of CC and cholesterol. The peaks at 2800- 3000 cm^{-1} (-CH_2 and -CH_3 groups), 3315 cm^{-1} (attributed to -OH stretching), 1636 cm^{-1} (side chain carbonyl group) and 1389 cm^{-1} (-$CH_2$and -CH_3 bending vibration) are shielded by phospholipids.

Percentage drug release:

The pure CC showed the slowest rate of dissolution, since at the ending of the dissolution period simply about 44% w/w of CC was suspended. The prepared phytosomes from the different extracts of CC, showed a considerably fast discharge of CC at the end point of dissolution period, i.e., over 89% w/w of CC.

Conclusion

It is cleared that phytosomal complexes are clearly formed. Results of various studies showed that, improved bioavailability and solubility is achieved by forming phytosomes. Consequently, it can be concluded that the phytosomes may be act as promising dosage form

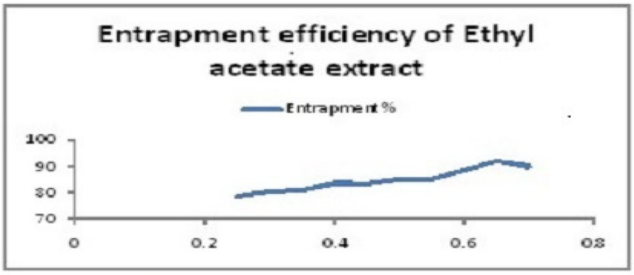


FIGURE 1- Entrapment efficiency of Ethyl acetate extract.

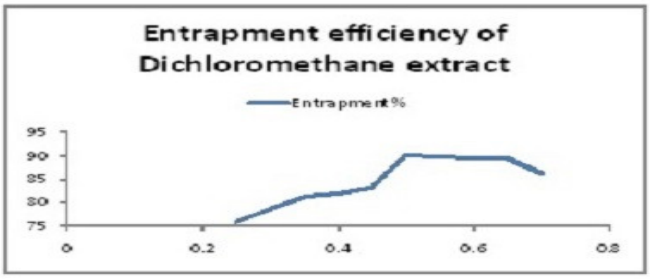


FIGURE 2- Entrapment efficiency of Dichloromethane extract.

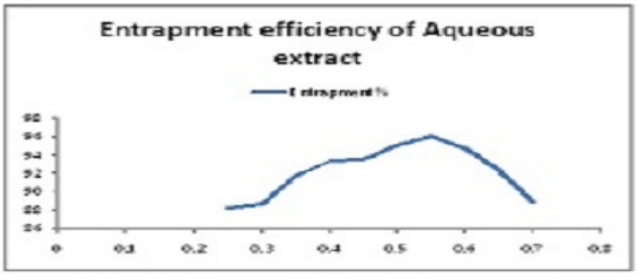


FIGURE 3- Entrapment efficiency of Aqueous extract.

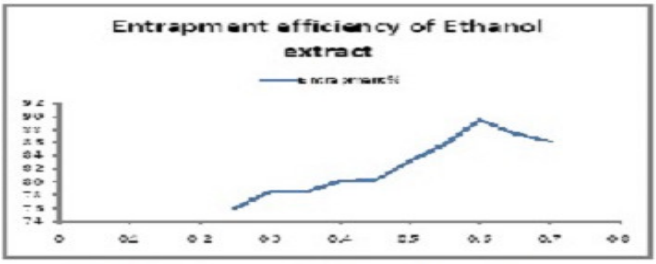


FIGURE 4- Entrapment efficiency of Ethanol extract.

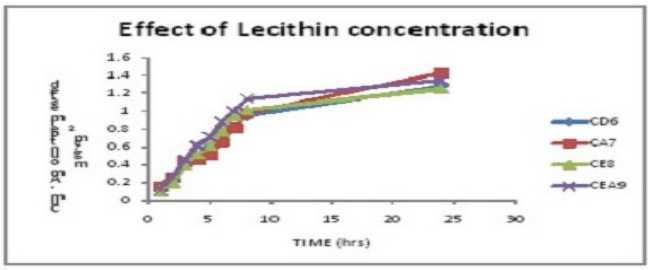


FIGURE 5- Effect of lecithin concentration

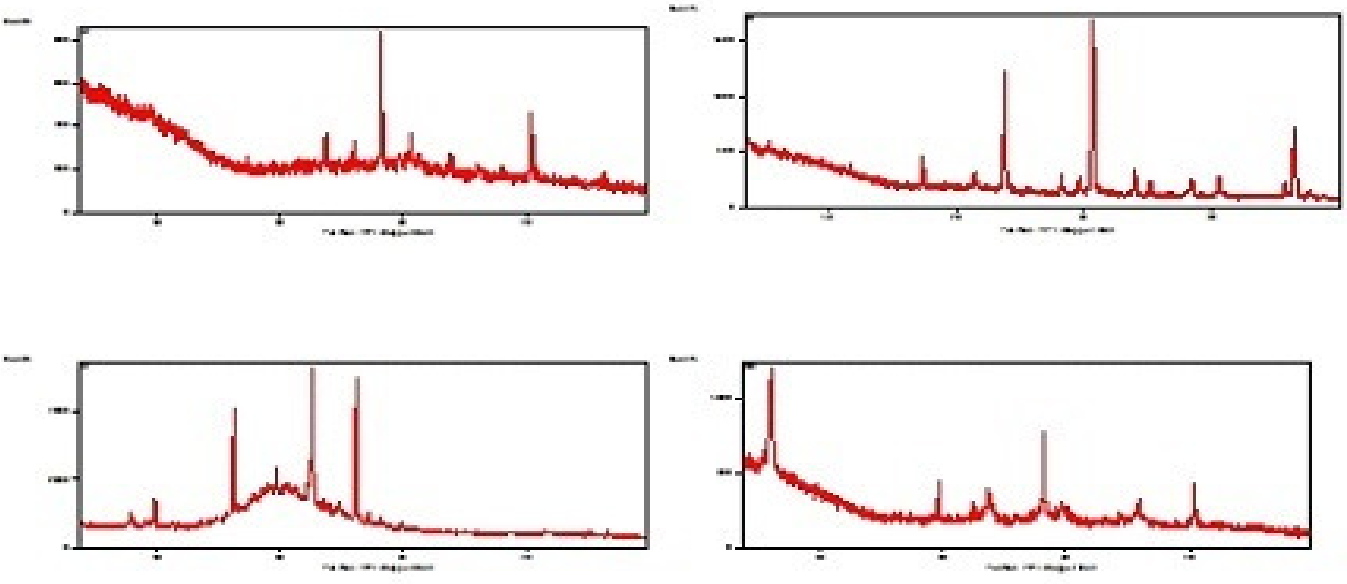


FIGURE 6- The x-ray diffraction (XRD) patterns of phytosomes

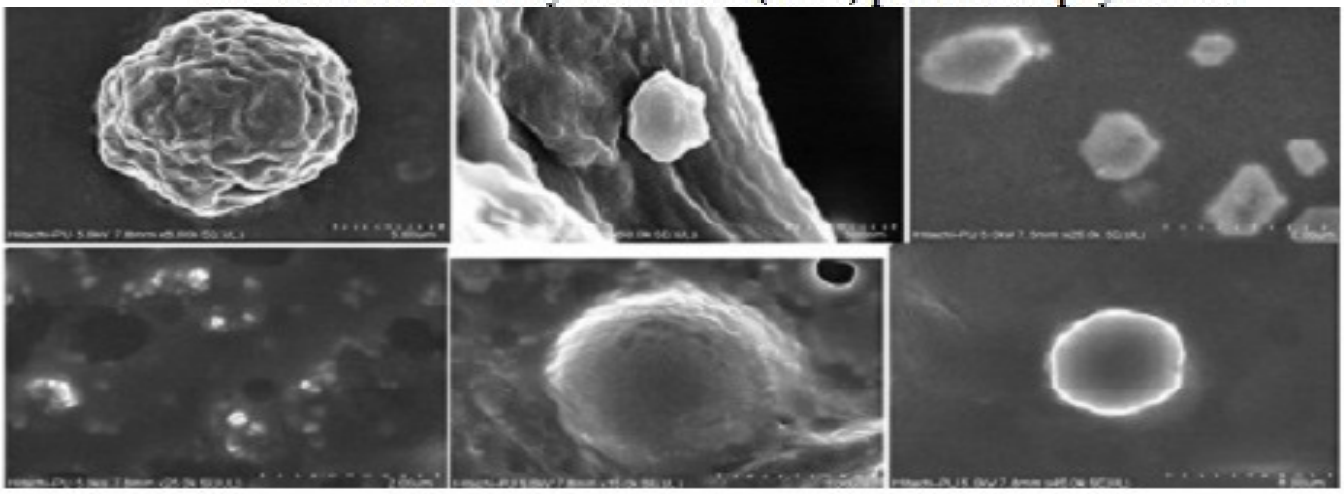
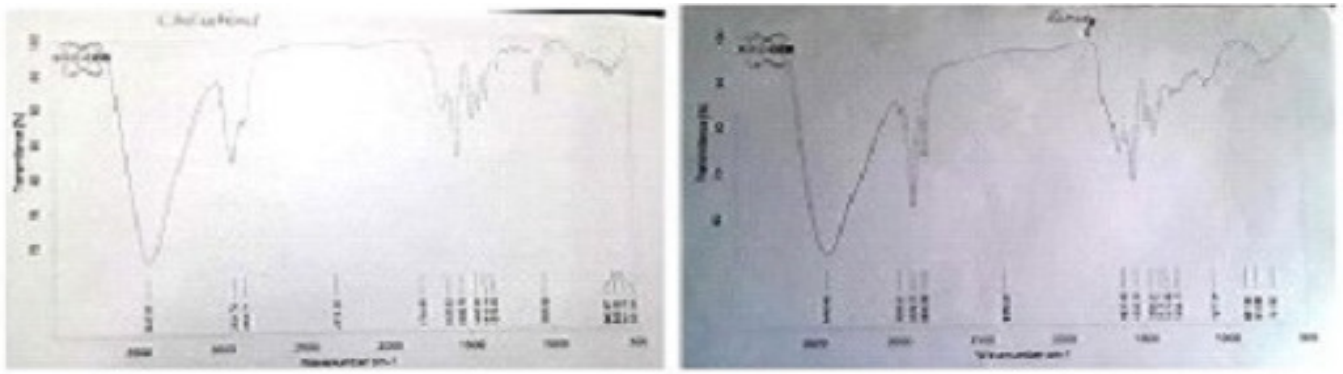


FIGURE 7- a, shows crystalline state of Citrullus Colocynth, b, shows Physical mixture (PM), c, d, e, and f shows CA7, CE8, CD6 and CEA9 respectively



FT-IR of cholesterol **FT- IR of Citrullus Colocthynthis**

FIGURE 8- FT-IR of Cholesterol and *Citrullus colocynthus*

Table I : Chemical tests for Preliminary screening

Chemical tests for Preliminary screening		
Alkaloids	Dragendorff's test	Compound furnished reddish brown precipitate
	Mayer's test	Compound gives cream color while reacted with the reagent, i.e., Potassium Mercuric iodide solution.
	Wagner's test	Compound was present with reddish brown precipitate while reacted with the reagent,
	Hager's test	Compound gives give yellow precipitate while reacted with the reagent, i.e., Saturated solution of picric acid.
	Picrolonic acid test	Compound gives yellow colour precipitate picrolonic acid.
Amino acids	Millon's test	Compound gives white precipitate while reacted with the reagent.
	Ninhydrine test	Compound gives violet precipitate while reacted with the reagent.
Carbohydrates	Molisch's test	α-naphthol + conc. sulphuric acid forms a violet colored ring with the compound in the test tube.
	Selivanoff's test (Test for ketones)	Resorcinol + conc. hydrochloric acid forms a rose colour with the compound.
	Test for pentoses	Phloroglucinol + conc. hydrochloric acid forms a red colour with the compound.
Flavonoids	Shinoda test	Few magnesium turnings + conc. hydrochloric acid forms a crimson red or pink colour with the compound.
	Alkaline reagent test	NaOH forms a yellow colour with the compound that turns colourless with the addition of dil. hydrochloric acid.
	Zinc hydrochloride test	Zinc dust + conc. hydrochloric acid forms a red colour with the compound.

Table II : Formulation composition of phytosomal formulation of aqueous extract of *Citrullus colocynthis*

S. No.	**Type of formulation**	**cholesterol**	**Lecithin**	**chloroform**	**extract of *Citrullus colocynthis***	**Cholesterol: lipid ratio**
1	CA1	0.5mg	0.25mg	5ml	5ml	0.5:0.25
2	CA2	0.5mg	0.3mg	5ml	5ml	0.5:0.3
3	CA3	0.5mg	0.35mg	5ml	5ml	0.5:0.35
4	CA4	0.5mg	0.4mg	5ml	5ml	0.5:0.4
5	CA5	0.5mg	0.45mg	5ml	5ml	0.5:0.45
6	CA6	0.5mg	0.5mg	5ml	5ml	0.5:0.5
7	CA7	0.5mg	0.55mg	5ml	5ml	0.5:0.55
8	CA8	0.5mg	0.6mg	5ml	5ml	0.5:0.6
9	CA9	0.5mg	0.65mg	5ml	5ml	0.5:0.65
10	CA10	0.5mg	0.7mg	5ml	5ml	0.5:0.7

Table III : Formulation composition of phytosomal formulation of ethyl acetate extract of *Citrullus colocynthis*

S. No.	**Type of formulation**	**cholesterol**	**Lecithin**	**chloroform**	**extract of *Citrullus colocynthis***	**Cholesterol: lipid ratio**
1	CEA1	0.5mg	0.25mg	5ml	5ml	0.5:0.25
2	CEA2	0.5mg	0.3mg	5ml	5ml	0.5:0.3
3	CEA3	0.5mg	0.35mg	5ml	5ml	0.5:0.35
4	CEA4	0.5mg	0.4mg	5ml	5ml	0.5:0.4
5	CEA5	0.5mg	0.45mg	5ml	5ml	0.5:0.45
6	CEA6	0.5mg	0.5mg	5ml	5ml	0.5:0.5
7	CEA7	0.5mg	0.55mg	5ml	5ml	0.5:0.55
8	CEA8	0.5mg	0.6mg	5ml	5ml	0.5:0.6
9	CEA9	0.5mg	0.65mg	5ml	5ml	0.5:0.65
10	CEA10	0.5mg	0.7mg	5ml	5ml	0.5:0.7

Table IV : Formulation composition of phytosomal formulation of ethanol extract of *Citrullus colocynthis*

S. No.	Type of formulation	cholesterol	Lecithin	chloroform	extract of *Citrullus colocynthis*	Cholesterol: lipid ratio
1	CE1	0.5mg	0.25mg	5ml	5ml	0.5:0.25
2	CE2	0.5mg	0.3mg	5ml	5ml	0.5:0.3
3	CE3	0.5mg	0.35mg	5ml	5ml	0.5:0.35
4	CE4	0.5mg	0.4mg	5ml	5ml	0.5:0.4
5	CE5	0.5mg	0.45mg	5ml	5ml	0.5:0.45
6	CE6	0.5mg	0.5mg	5ml	5ml	0.5:0.5
7	CE7	0.5mg	0.55mg	5ml	5ml	0.5:0.55
8	CE8	0.5mg	0.6mg	5ml	5ml	0.5:0.6
9	CE9	0.5mg	0.65mg	5ml	5ml	0.5:0.65
10	CE10	0.5mg	0.7mg	5ml	5ml	0.5:0.7

Table V : Formulation composition of phytosomal formulation of dichloromethane extract of *Citrullus colocynthis*

S. No.	Type of formulation	cholesterol	Lecithin	chloroform	extract of *Citrullus colocynthis*	Cholesterol: lipid ratio
1	CD1	0.5mg	0.25mg	5ml	5ml	0.5:0.25
2	CD2	0.5mg	0.3mg	5ml	5ml	0.5:0.3
3	CD3	0.5mg	0.35mg	5ml	5ml	0.5:0.35
4	CD4	0.5mg	0.4mg	5ml	5ml	0.5:0.4
5	CD5	0.5mg	0.45mg	5ml	5ml	0.5:0.45
6	CD6	0.5mg	0.5mg	5ml	5ml	0.5:0.5
7	CD7	0.5mg	0.55mg	5ml	5ml	0.5:0.55
8	CD8	0.5mg	0.6mg	5ml	5ml	0.5:0.6
9	CD9	0.5mg	0.65mg	5ml	5ml	0.5:0.65
10	CD10	0.5mg	0.7mg	5ml	5ml	0.5:0.7

Table VI : Organoleptic Characters of *Citrullus colocynthis*

Organoleptic Characters of Citrullus Colocynthis			
Size	5-8 cm in diameter	Appearance	Smooth external surface, filled with spongy white pulp with multiple seeds.
Shape	spherical	Taste	bitter
colour	Yellow-green	odour	Characteristic

Table VII : Results Preliminary Phyto-chemical screening of Different Extracts of *Citrullus colocynthis*

Results Preliminary Phyto-chemical screening of Different Extracts of Citrullus colocynthis				
	Ethyl acetate extract	**Aq. Extract**	**Dichloromethane extract**	**Ethanolic extract**
Test for Alkaloids	-	-	-	-
Tests for Proteins and Amino acids	+	+	+	+
Tests for carbohydrates	+	+	+	+
Test for steroids and triterpenoids	+	+	+	+
Test for flavonoids	+	+	+	+
Note: + : Present; - : Absent.				

Table VIII : Total flavonoid content of CC (mg QE/g)

Solvents extract	**TFC of CC (mg QE/g)**
Ethyl acetate	46.34±0.03
Dichloromethane	28.7±1.04
Aqueous	48.32±0.13
Ethanol	59.84±0.90

Table IX : TLC results of *Citrullus colocynthis* extract

TLC results of Citrullus colocynthis extract				
	Ethyl acetate extract	**Aq. Extract**	**Dichloromethane extract**	**Ethanolic extract**
U. V. Light (366nm) / Fluorescent Zones	4-Spots at Rf -0.27 (Red), 0.65 (Light sky Blue), 0.86 (Sky Blue), 0.94 (Red & sky Blue).	3-Spots at Rf-0.25, 0.43, 0.75 (all blue).	4-Spots at Rf -0.30, 0.45, 0.55 and 0.86 (all blue).	2-Spots at Rf -0.36 and 0.74 (all blue).
Exposure to Iodine Vapour	4-Spots at Rf -0.27, 0.65, 0.94, and 0.86 (All Yellow)	4-Spots at Rf -0.25, 0.35, 0.43, and 0.75 (All Yellow)	4-Spots at Rf -0.30, 0.45, 0.55 and 0.86 (All Yellow)	2-Spots at Rf -0.36 and 0.74 (All Yellow)

Table X : Effect of lecithin concentration

Formulation		**Vesicle Size (µm)**	**Entrapment (%)**	**Cumulative amount of drug permeated (mg/cm^2)**
Ethyl acetate	CEA1	6.0 ± 0.131	78.49 ± 0.85	1.2801 ± 0.031
	CEA2	6.2± 0.138	80.26 ± 0.60	1.3236 ± 0.058
	CEA3	6.5 ± 0.184	81.03± 0.48	1.3836 ± 0.026
	CEA4	6.5 ± 0.0064	83.64 ± 0.56	1.3960 ± 0.085
	CEA5	6.8 ± 0.09	83.26 ± 0.70	1.4052 ± 0.048
	CEA6	6.8 ± 0.267	85.00 ± 0.85	1.1484 ± 0.86
	CEA7	7.0 ± 0.057	85.03± 0.449	1.4236 ± 0.026
	CEA8	7.0 ± 0.0064	88.64 ± 0.56	1.3460 ± 0.025
	CEA9	7.0 ± 0.09	92.26 ± 0.70	1.3452 ± 0.048
	CEA10	7.8 ± 0.048	90.10 ± 0.758	1.0530 ± 0.076
Dichloromethane	CD1	6.0 ± 0.074	76.00 ± 0.873	1.2406 ± 0.035
	CD2	6.2± 0.138	78.46 ± 0.488	1.2890 ± 0.093
	CD3	6.5 ± 0.184	81.48± 0.657	1.2794 ± 0.067
	CD4	6.5 ± 0.0064	82.20 ± 0.731	1.2779 ± 0.052
	CD5	6.8 ± 0.09	83.26 ± 0.70	1.3028 ± 0.064
	CD6	7.8 ± 0.048	90.10 ± 0.758	1.0530 ± 0.076
	CD7	7.0 ± 0.073	89.80± 0.65	1.3215 ± 0.083
	CD8	7.0 ± 0.085	89.58 ± 0.70	1.3913 ± 0.079
	CD9	6.8 ± 0.054	89.49 ± 0.54	1.5012 ± 0.086
	CD10	6.0 ± 0.061	86.20 ± 0.450	1.2721 ± 0.049
Aqueous	CA1	6.8 ± 0.465	88.23 ± 0.61	1.5809 ± 0.062
	CA2	6.8± 0.159	88.65 ± 0.38	1.5286 ± 0.036
	CA3	7.0 ± 0.134	91.64± 0.28	1.5866 ± 0.060
	CA4	7.5 ± 0.0064	93.36 ± 0.41	1.4990 ± 0.065
	CA5	7.8 ± 0.09	93.62 ± 0.69	1.3620 ± 0.062
	CA6	7.8 ± 0.267	95.05 ± 0.37	1.2694 ± 0.069
	CA7	7.0 ± 0.063	96.08± 0.658	1.2439 ± 0.052
	CA8	6.8 ± 0.094	94.84 ± 0.37	1.2610 ± 0.094
	CA9	6.6 ± 0.056	92.34 ± 0.64	1.2405 ± 0.062
	CA10	7.8 ± 0.048	88.90 ± 0.587	1.1530 ± 0.074
Ethanol	CE1	6.0 ± 0.062	76.00 ± 0.743	1.2956 ± 0.642
	CE2	6.2± 0.164	78.46 ± 0.268	1.2235 ± 0.125
	CE3	6.5 ± 0.199	78.48± 0.956	1.2684 ± 0.241
	CE4	6.5 ± 0.003	80.20 ± 0.342	1.2698 ± 0.140
	CE5	6.8 ± 0.61	80.26 ± 0.215	1.3501 ± 0.360
	CE6	7.0 ± 0.835	83.10 ± 0.785	1.1052 ± 0.364
	CE7	7.0 ± 0.161	85.80± 0.695	1.357 ± 0.046
	CE8	7.2 ± 0.549	89.58 ± 0.104	1.3369 ± 0.410
	CE9	7.0 ± 0.310	87.49 ± 0.520	1.4357 ± 0.240
	CE10	6.8 ± 0.260	86.20 ± 0.218	1.3594 ± 0.026

Table XI : Effect of Lecithin Concentration on cum. amount of drug permeated

Time (hrs)	Cumulative amount of drug permeated (mg / cm^2)			
	CD6	**CA7**	**CE8**	**CEA9**
1	0.1270 ± 0.032	0.1592 ± 0.058	0.1074 ± 0.048	0.1210 ± 0.067
2	0.2603 ± 0.095	0.2619 ± 0.094	0.1819 ± 0.098	0.2624 ± 0.037
3	0.4010 ± 0.047	0.4338 ± 0.054	0.3954 ± 0.062	0.4541 ± 0.046
4	0.4836 ± 0.068	0.4545 ± 0.061	0.5137 ± 0.054	0.6159 ± 0.042
5	0.6016 ± 0.046	0.5074 ± 0.029	0.6069 ± 0.031	0.7105 ± 1.004
6	0.6922 ± 0.051	0.6567 ± 0.082	0.7782 ± 0.094	0.8777 ± 0.031
7	0.8181 ± 0.083	0.8133 ± 0.047	0.9403 ± 0.054	1.0003 ± 0.085
8	0.9489 ± 0.049	0.9753 ± 0.091	1.009 ± 0.082	1.1389 ± 0.049
24	1.2801 ± 0.031	1.4236 ± 0.085	1.2460 ± 0.025	1.3452 ± 0.048

Table XII : Aqueous Solubility (µg/ ml)*

S.No.	Sample	Aqueous Solubility (µg/ ml)*	n-Octanol solubility (µg/ ml)*
1	Citrullus Colocynthis (CC)	4.45±0.33	3.5.65±0.54
2	Physical mixture (PM)	8.65±1.23	432.21±0.04
3	CEA9	85±0.09	617.34±0.58
4	CE8	76.4±0.65	564.85±0.29
5	CD6	62.7±0.56	635±0.32
6	CA7	89.4±0.04	725±0.51

Acknowledgement

The authors are gratefully acknowledged to the Director and Management of Chitkara College of Pharmacy, Chitkara University (Pb.) for facilities provided during the study.

This research did not receive any specific grant from funding agencies in the public, commercial, alternatively, not-for-profit sectors.

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