Journal of Pharmaceutical Research International

33(57A): 369-382, 2021; Article no.JPRI.77932
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Preparation and Evaluation of Nanophytosomes of Aqueous Extract of Leaves of *Momordica charantia*

P. Ramakrishna Reddy [a*#], V. Sreedhar [a], K. Rajesh Reddy [a], D. Murali [a] and K. Sudhakara [a]

[a] Department of Pharmaceutics, Balaji College of Pharmacy, Rudrampeta, Ananthapuramu, Andhra Pradesh, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i57A34008

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77932

Original Research Article

Received 06 October 2021
Accepted 12 December 2021
Published 14 December 2021

ABSTRACT

Though there was not enough data available throughout the phytosome research, authors tried maximum to provide all inputs for the preparation of phytosomes. The objectives of the present research work focused on the investigation of phyto chemical constituents of aqueous extract, preparation of nanophytosomes of aqueous extract. *Momorica charantia* plants were collected locally from the village of Muhavur. The leaves were separated from the plant and the leaves were washed with water and then again washed with chloroform to remove soil particles and the leaves were spread and dried in the shade for 4 days. The aqueous extract of *Momorica charantia* obtained was subjected to qualitative analysis to test the presence of various phytochemicals. Particle size of prepared nanophytosomes was analyzed by photon correlation spectroscopy using a Shimadzu particle size analyzer (SALD 2101, Japan). Diluted nanophytosomal suspension was placed into the sample dispersion unit while stirring at room temperature (in order to reduce the inter particle aggregation). All analyses has been performed in triplicate. Nanophytosomes of *Momordica charantia* aqueous extract was effectively prepared and tested. The aqueous extract was evaluated phyto chemical screening followed by all characterization studies. Phytochemical screening study remaining that the extract consists of flavanoids. The characterization study showed that the phytosomes are having nano size, good stability properties with round to spherical shape with smooth surfaces.

Keywords: Flavanoids; Momordica charantia; nanophytosomes; phytochemical screening.

[#]Professor;
**Corresponding author: E-mail: dr.reddyrk@gmail.com;*

1. INTRODUCTION

Momordica charantia has a non-nitrogenous neutral principle charantin, and on hydrolysis gives glucose and asterol. The fruit pulp of *Momordica charantia* has soluble pectin. Galactouronic acid is also obtained from the pulp [1-3]. *Momordica charantia* fruits glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. The presence of anun identified alkaloid and 5-hydroxytryotamine is also reported. The 5HT content is reported to be present [4-6]. Theether extract residue of the alcoholic concentrate from the leaves of *Momordica charantia* is reported to reveal hypoglycemic activity comparable to that of tolbutamide. The protein termed as P-insulin extracted from fruits in crystalline form is also tested. In India, diverse parts of the plant are used as claimed treatments for diabetes and as a antibilious, emetic, stomachic, laxative, anthelmintic agent, for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcer, gout, and rheumatism. It has a number of purported uses including cancer prevention, treatment of diabetes, fever, HIV and AIDS, and infections [7-9]. For cancer prevention, HIV and AIDS, and treatment of infections, there is preliminary laboratory research, but no clinical studies in humans showing a benefit. In 2017, the University of Peradeniya researchers revealed that bitter gourd seeds can be potentially used to destroy cancer cells and they were successfully administered to patients in Kandy General Hospital Cancer Unit. The Memorial Sloan Kettering Cancer Center concludes that bitter melon "cannot be recommended as a replacement therapy for insulin or hypoglycaemic drugs" [10-13].

1.1 Phytosome Technology and its Advantages

Hydrophilic phytoconstituents has the ability to bind with phospholipids. A specified amount of phospholipid (phosphatidylcholine) react with the herbal extract in a non- polar solvent. The phospholipid (phosphatidylcholine) used in this formulation was obtained from soybean with both lipophilic (phosphotidyl part) and hydroplilic (choline) portions. The body portion has choline group which is hydrophilic and the tail portion has phosphotidyl group which is lipophilic in nature, thereby the hydrophilic group is encoded within the lipophilic group to form a stable complex, phytosomes are formed [14-17]. The bonds formed are chemical in nature, which in additional provides better stability for the drug molecule in complex with wide range of advantages. Phosphatidyl choline used in this formulation has dual function, it act as a carrier for drug moiety with nutritional value [18].

1.2 Preparation of Nanophytosomes

Though there was not enough data available throughout the phytosome research, authors tried maximum to provide all inputs for the preparation of phytosomes. The method for the preparation of phytosomes is as follows: In the first step, phospholipids are obtained from either natural or synthetic sources are to be dissolved in a organic solvent such as acetone or dioxane. To the solution of phospholipids, herbal extract is added with constant stirring. Then the solution is allowed to evaporate on a spray dryer. The ratio between the portions in the range of 0.5 to 2.0 moles but the most preferable ratio is1:1. Thin flim is formed after evaporation of the solvent. Futher hydration of the flim leads to formation of phytosomal suspension. The formed phytosomes will be collected by precipitation technique. The collected phytosomes are futher subjected to drying by lyophilisation method. The objectives of the present research work focused on the investigation of phyto chemical constituents of aqueous extract, preparation of nanophytosomes of aqueous extract [19-21].

2. MATERIALS AND METHODS

2.1 Materials

Cholesterol were obtained from Loba Chem lab pvt ltd, Maharashtra. Phosphotidyl Choline is obtained from Lipoid pharma pvt ltd, Germany. Chloroform was obtained from Sisco Research lab, pvt ltd, Mumbai. All reagents and glass ware used are of analytical grade.

2.2 Methodology

2.2.1 Collection and processing of *Momordica charantia* plant material

Momorica Charantia plants were collected locally from the village of Muhavur, Srivilliputhur (Virudhunagar Dist,Tamilnadu). The leaves were separated from the plant and the leaves were washed with water and then again washed with chloroform to remove soil particles and the leaves were spread and dried in the shade for 4 days.

2.2.2 Preparation of aqueous extract of *Momordica charantia*

The *Momordica charantia* leaves were subjected to size reduction by trituration by using mortar and pestle to make into fine powder. Weigh 10g of powder and it is dissolved in a 250ml of boiling water. Then the mixture of powder and water is placed in water bath at 400c for 1 hour. Then it is filtered by using whatmann filter paper. Then the filtrate was concentrated on water bath at 400c for 2days.Then finally extract was collected and stored in desiccator at room temperature.

2.2.3 Preliminary phytochemical analysis [22-25]

The aqueous extract of momorica charantia obtained was subjected to qualitative analysis to test the presence of various phytochemicals like alkaloids, flavonoids, steroids, phenols, proteins and amino acids, terpenoids, anthraquinones and quinones etc. Preliminary phytochemical analysis for momordica charatia leaves extracts were carried out as per the protocol mentioned in Harbore,1998 (Paterson, 1999). For HPTLC (silica gel G 60F254 TLC plates of E. Merck, layer thickness 0.2 mm) fingerprint analysis was established for aqueous extracts of *Momordica charantia* leaves. HPTLC was performed on (10 cm X 10 cm) aluminum backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of quercetin and test were applied to the plates as bands 8.0 mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by use of a Camag Linomat V sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28±2oc), with toluene: ethyl acetate: formic acid [(5: 4: 1) (v/v/v)], as mobile phase, in a Camag glass twintrough chamber previously saturated with mobile phase vapour for 20 min. Quercetin of (100 µg/mL) was used as standard (Vijayalakshmi, Ravichandiran, Malarkodi, Nirmala, & Jayakumari, 2012).

2.2.4 Preparation of nanophytosomes: Thin film hydration method [26-28]

Accurately weighed quantity of egg lecithin and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and sonicated for 10 min using bath sonicator. Organic solvent removal is prepared by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with Aqueous extract of momorica charantia leaves in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract was sonicated for 40 minutes in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8 0C) until used. The different phytosome complexes of momorica charantia F1, F2, F3 & F4 containing molar ratio of 1:0.5:1, 2:1:1, 1:0.5:2 and 2:1:2 of Egg lecithin, Cholesterol and Momorica Charantia were prepared.

2.3 Characterization of Nanophytosomes

2.3.1 Particle size

Particle size of prepared nanophytosomes was analyzed by photon correlation spectroscopy using a Shimadzu particle size analyzer (SALD 2101, Japan). Diluted nanophytosomal suspension was placed into the sample dispersion unit while stirring at room temperature (in order to reduce the inter particle aggregation). All analyses has been performed in triplicate.

2.3.2 Zeta potential determination

Surface charge of momorica charantia -loaded nanophytosomes was determined using a Malvern Zetasizer (Nano-ZS, UK). Samples were diluted (50 folds) using distilled water and then analysis was performed at 25 ºC and 149 watt. The average three zeta potential determinations of the nanophytosomes were premeditated.

2.3.3 Fourier transformer-infra red spectroscopy (FTIR) analysis

FT-IR spectral data can be taken to determine the structure and chemical stability of pure drug in the presence of excipients, physical mixture of egg lecithin and cholesterol, physical mixtures and nanophytosomal formulation. were evaluated by FT-IR analysis. The spectroscopic evaluation of the formed complex can be confirmed by FTIR simply by mparing the spectrum of the complex and the individual components and that of the mechanical mixtures. Samples were mixed with dry crystalline KBr in a ratio of 1:100 and pellets were prepared. The mixture was grounded or triturated into fine powder using an agate mortar before compressing into KBr disc. Each KBr disc was scanned at 4 mm/s at a resolution of 2. FTIR can also be considered as a valuable tool in

confirming the stability of the phytosomal complex. FT-IR spectra were obtained using a FT-IR spectrometer. Spectral scanning can be done in the range between 4000-400 cm-1.

2.3.4 Differential scanning calorimetry (DSC) analysis

Thermodynamical techniques are applied for determining the thermal stress of medicinal compounds of the excipients as well as their interactions during the formulation process. The thermal analysis of the *Momordica charantia* , physical mixture of egg lecithin and cholesterol, physical mixture of egg lecithin and cholesterol and extract of momorica charantia were placed in the aluminum crimp cell and heated at 100C/min from 0 to 4000C in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer. Momorica charantia leaves extract, phospholipon and phytosome were placed in the aluminum crimp cell and heated at 100C/min from 0 to 400 0C in the atmosphere of nitrogen (TA Instruments, USA, Model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer.

2.3.5 Scanning electron microscopy (SEM) analysis

Scanning electron microscopy has been used to determine particle size distribution and surface morphology of the complexes. Samples were studied using JEOL JSM- 6360 Scanning microscope (Japan). Approximately 5 µL of the nanophytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the particle size of the formulation was viewed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss). The particles were coated with platinum by using vaccum evaporator and thus, the coated samples were viewed and photographed in JEOL JSM-6701F Field Emission SEM. Digital images of phytosome complex of momorica charantia were taken by random scanning of the stub at different magnifications.

2.3.6 X-ray diffraction (XRD) analysis

XRD is a unique method in determination of crystallinity of a compound and when properly interpreted, by comparison with drug XRD pattern before formulation., allows the identification of the drug crystalline changes. XRD was done on pure extract, physical mixtures of egg lecithin and cholesterol, physical mixtures and nanophytosome to see the crystallinity in the substance. Sample was scanned in the angular range of 50 - 800 in a PHILIPS XPert Pro X-Ray Diffractometer. Dried powder sample was kept in sample holder (20 mm × 15mm × 2mm) which was fitted into the instrument and X-ray was passed through the sample.

2.3.7 Transmission electron microscopy (TEM) analysis

Vesicles morphology of nanophytosome was observed visually with a JEOL JEM 1400 (Japan) Transmission Electron Microscopy (TEM). A total volume of 10 ml sample was dispersed before the sample was analyzed. The mixture was then stirred and a drop of the sample was placed on the specimen. The 400 mesh grid was placed over the specimens and allowed to stand for 1 minute. Residual droplets on the grid were cleaned using a filter paper. A drop of 5 uranyl acetate was dropped over the grid and the rest of the excess solution was removed using a filter paper. The grid was left for 30 minutes and the films were then viewed on a transmission electron microscope and photographed.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Investigation

The results of the phytochemical study were tabulated in Table-1. The phytochemical screening of the aqueous extract of *Momordica charantia* leaves revealed the presence of steroids, flavonoids, alkaloids, sterols, phytosterols, terpenoids, tannins, proteins and amino acid, phenols, saponins. Tannins were absent in aqueous extract of *Momordica charantia* [29-30].

3.1.1 HPTLC finger printing analysis of aqueous extract of *Momordica charantia* leaves

Preliminary phytochemical investigation divulges the presence of glycosides, phenolic compounds, flavonoids, proteins, amino acids and saponins in aqueous extracts of *Momordica charantia* . Hence, *Momordica charantia leaves* extract containing higher alttitude of phytoconstituents which may possibly take part in reactions in effective reduction of nanophytosomess. (Martinez-Perez et al., 2014). However, HPTLC

finger print analysis also confirms the presence of MCAE (Fig. 1) flavonoid which has influenced the conversion of nanophytosomes due to easily oxidizable conjugated hydroxyl groups in the molecule (Terenteva, Apyari, Dmitrienko, & Zolotov, 2015) [31-32].

Table 1. Preliminary phytochemical screening of *Momordica charantia* leaves extract

S. No.	Test	Results
1.	Test for Alkaloids	
	A. Mayer's test	+
	B. Wagner's test	+
	C. Hager's test	+
2.	Test for Flavanoids	
	A. NaoH test	+
	B. H2SO4	+
3.	Test for Sterols	
	A. Liebermann-Burchard test	+
4.	Test for Phytosterols	
	A. Salkowski test	+
5.	Test for Terpenoids	
	A. Liebermann-Burchard test	+
6.	Test for Protein and Amino Acid	
	A. Ninhydrin(aqueous)	+
	B. Ninhydrin(acetone)s	+
	C. Biuret test	+
7.	Test for Anthraquinones	
	A.Borntrager's test	+
8.	Test for Phenols	
	A. Ferric chloride test	+
	B. Liebermann test	+
9.	Test for Tannins	
	A. Ferric chloride test	-
10.	Test for Saponins	
	A. Foam test	+

(+) indicates positive reaction (-) indicates negative reaction

3.1.2 Characterization of MCAE nanophytosomes particle size

Particle size plays an important role in the stability, availability and organoleptic properties of the solution and the particles with smaller size is desirable. Results of particle size analysis indicated that nanophytosomes prepared with MCAE and PC possess the particle size in the average range of 584.1nm. Vesicle size tends to increase with increasing concentration of the complex. When the concentration of particles is too high, physical interaction either collision or electrostatic between vesicle is more pronoun. These interactions alter the movement of the particles and produce vesicles with a larger size. The high lipid composition in the formulation also increases the tendency for the formation of agglomerates, resulting in the bigger size of the vesicles. Polydispersity index is a measure of the heterogenecity of sizes of particles in a mixture *Momordica charantia* nanophytosomes prepared show polydispersity index value of 0.4 [33-35].

3.2 Zeta Potential

The Zeta potential is the electric potential in the interface or particle surface and is used to predict the stability of colloidal systems. Colloids with high absolute Zeta potential values (normally above 30 mV), regardless of their positivity or negativity, are electrically stabilized and those with low Zeta potential values are not stable and tend to coagulate or flocculate. In general, higher Zeta potential values indicate a higher and longer-term stability of the particles. Several factors such as pH, ionic strength, type and concentration of the used biopolymers are effective on the Zeta potential of the particles. The surface charge analysis results (-23.8 mV) and point to the high physical stability of MCAE nanophytosomes [36].

3.3 Fourier Trasform-Infra Red Spectroscopy (FT-IR) Studies

Spectroscopic analysis was used in order to identify and diagnose of complex formation between PC and extract. In FTIR spectroscopy, functional groups and their numbers were identified from the frequency of radiation that absorbs infrared spectra which showed the main chemical groups in extract and PC as well as the formation of new interactions between them in the nanophytosomes preparation process. The FTIR spectroscopy of *Momordica charantia* leaf extract and excipients are shown in Figs. 4, 5, 6. (Fig. 4) shows that the characterstic O-H peak at 3300.20cm^{-1}, C≡N peak at 2357.01 cm^{-1}, C≡C peak at 2169.92 cm^{-1}, C=C peak at 1988.61 cm^{-1}, C=C peak at 1635.64 cm^{-1}.(Fig. 6) shows that the C-H peak at 3020.53 cm^{-1}, C=C peak at 1988.61 cm^{-1}, C=O peak at 1734.01 cm^{-1}, C-N peak at 1215.15 cm^{-1},O-H peak at 927.76 cm^{-1}, C-H peak at 744.52 cm^{-1}, C-Br peak at 667.37 cm^{-1}. (Fig. 6) shows that the characterstic O-H peak at 3302.13 cm^{-1}, C≡ N peak at 2360.87 cm^{-1}, C≡C peak at 2167.99cm^{-1}, C=C peak at 1990.54 cm^{-1}, C=C peak at

1635.64 cm^{-1}. Hence there is no apperence of new peaks and disapperence of existence peaks in the presence of excipients indicates the MCAE and excipients are more compatible [37].

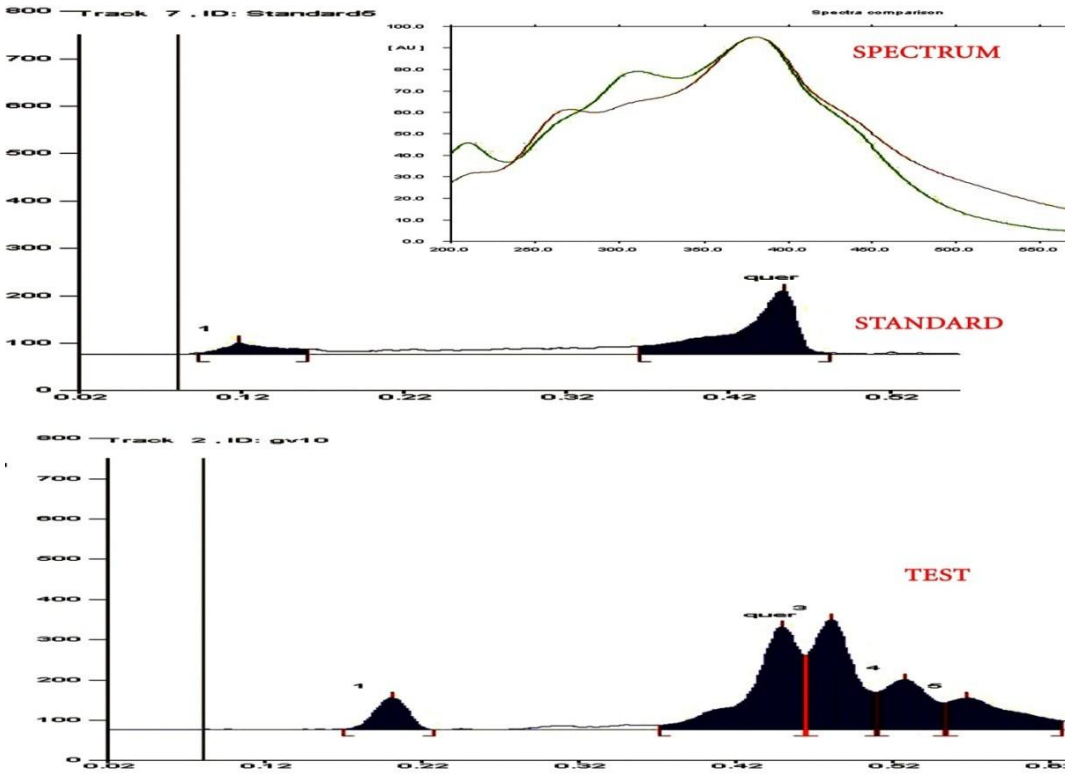

Fig. 1. HPTLC finger printing analysis of aqueous extract of *Momordica charantia* leaves

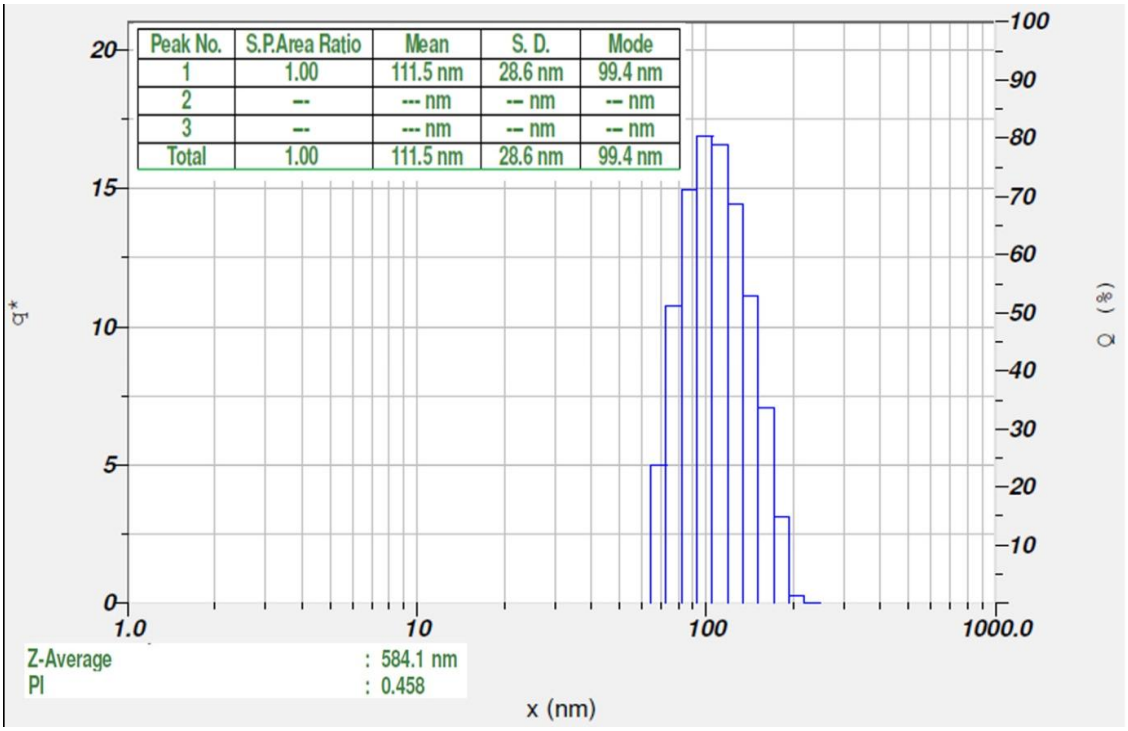

Fig. 2. Particle size of aqueous extract *Momordica charantia* nanophytosomes

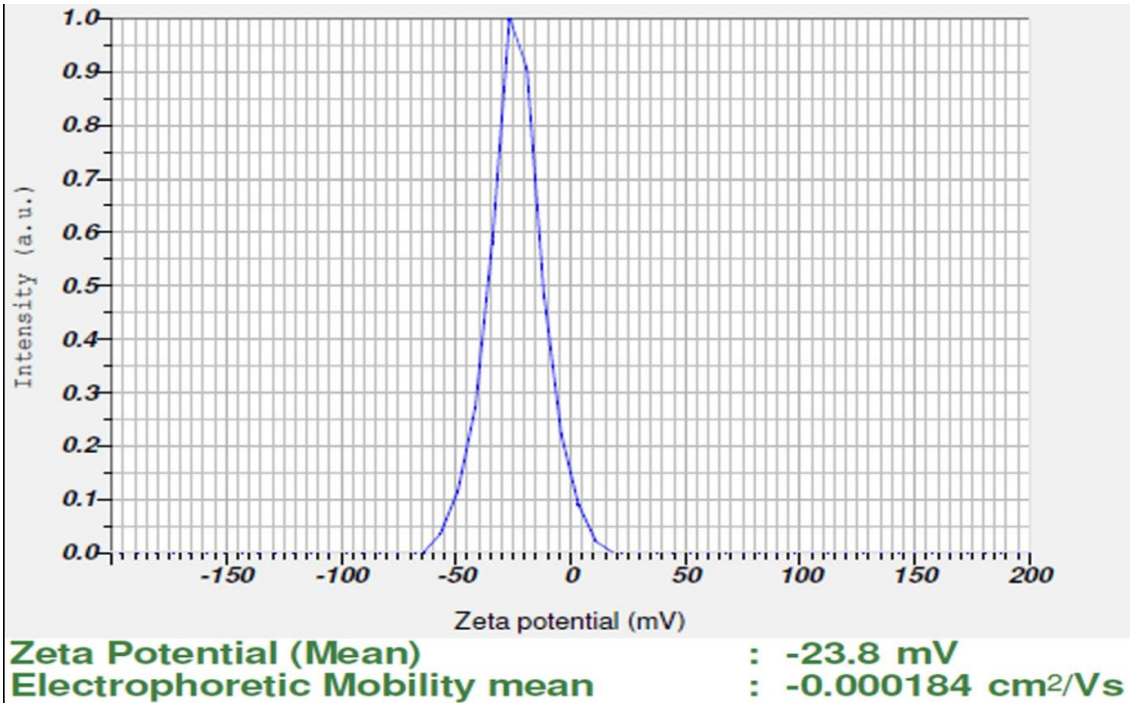

Fig. 3. Zeta potential distribution

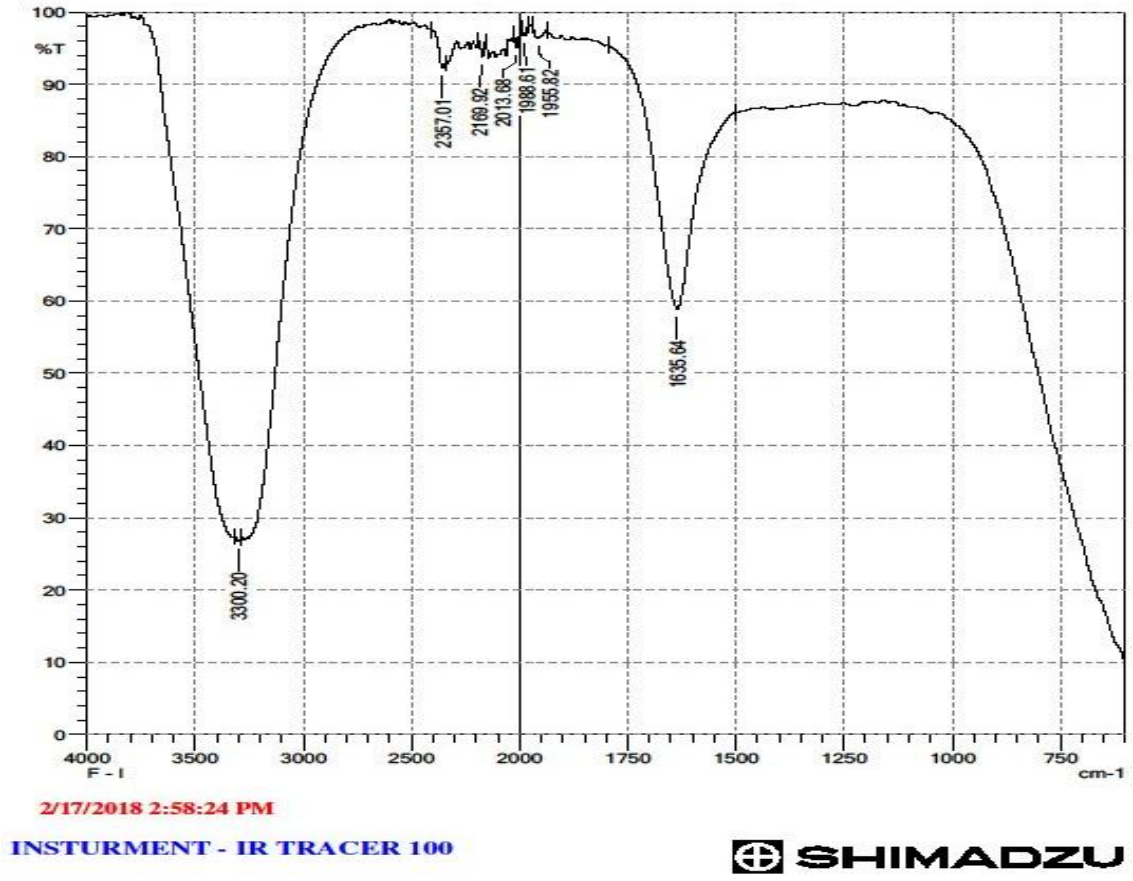

Fig. 4. FT-IR studies of *Momordica charantia* aqueous extract

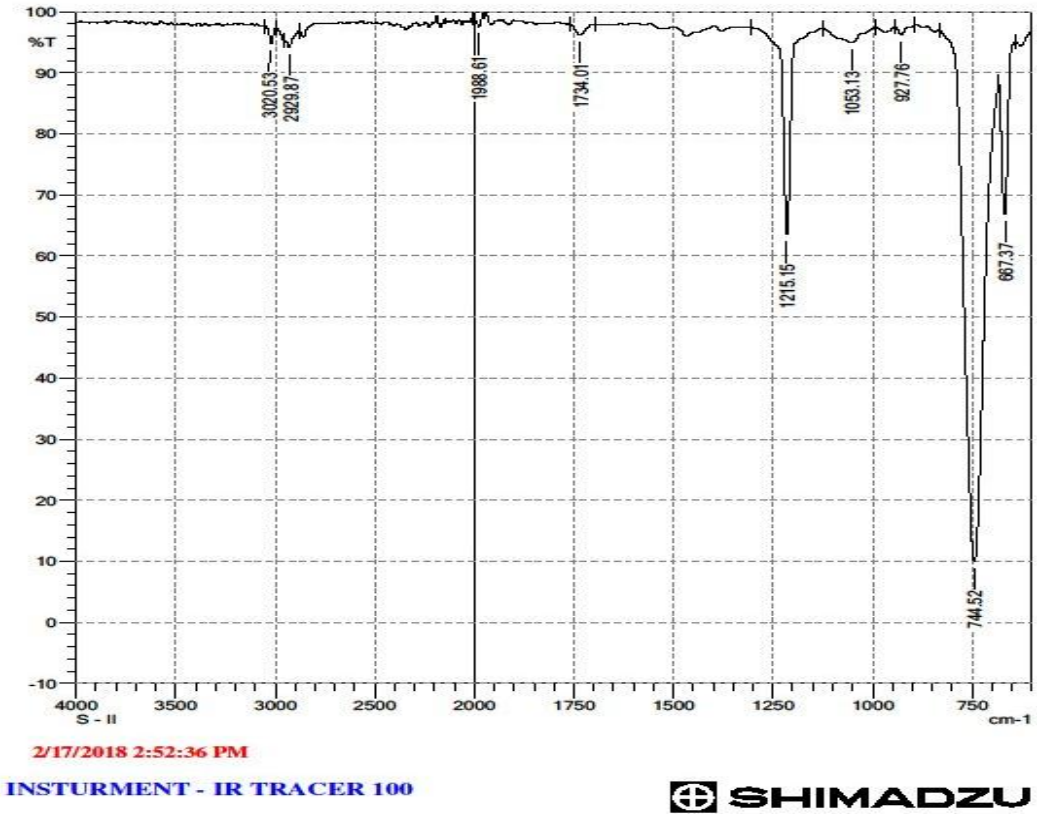

Fig. 5. FT-IR studies of phosphodityl-choline with cholesterol

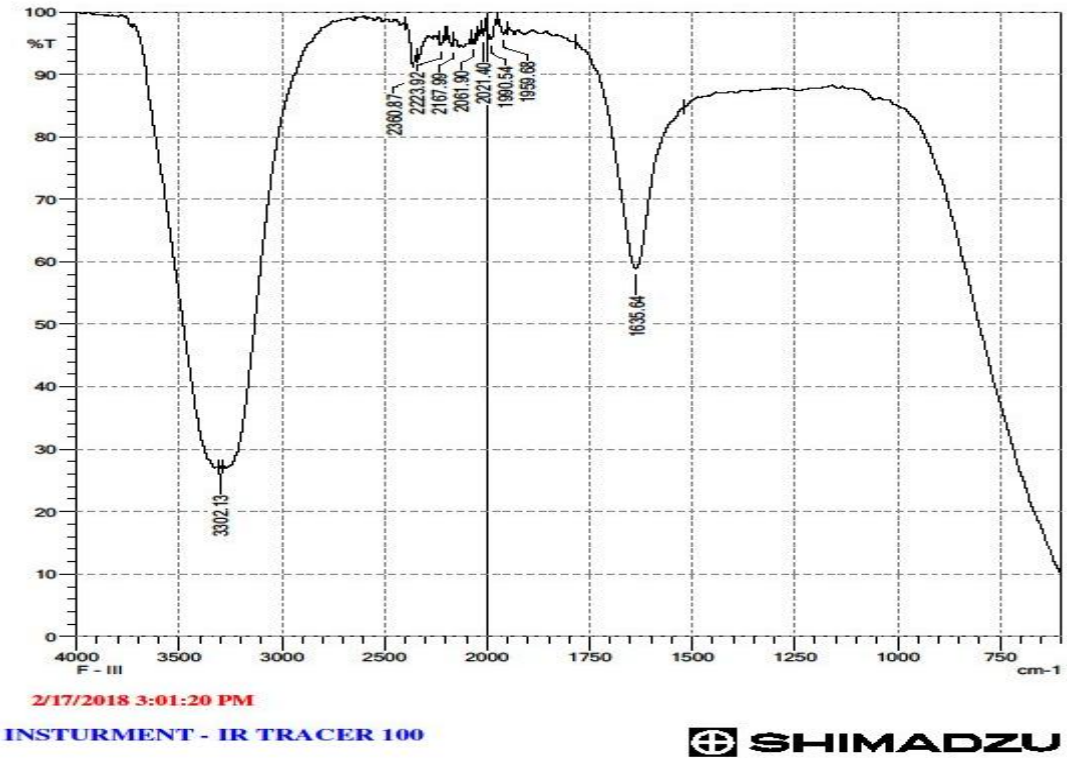

Fig. 6. FT-IR studies of *Momordica charantia* aqueous extract with excipients

Table 2. Interpretation of FT-IR studies

SNo.	Functional group	Wavenumber(cm^{-1})			
		Reference	Extract	Placebo	Extraxt+placebo
1.	O-H Stretch(carboxylic acid)	3300-2500	3300.20	_	3302.13
2.	CΞN stretch(nitriles)	2260-2210	2357.01	_	2360.87
3.	CΞN stretch(alkynes)	2260-2100	2169.92	_	2169.99
4.	C=Cstretch(alkenes)	1900-2000	2013.68	1988.61	2021.40
5.	C=Cstretch(alkenes)	1900-2000	1988.61	_	1990.54
6.	C=Cstretch(alkenes)	1900-2000	1955.82	_	1959.68
7.	C=Cstretch(alkenes)	1640-1680	1635.64	_	1635.64
8.	C-Hstretch(aromatic)	3100-3000	_	3020.53	_
9.	C-Hstretch(alkenes)	3100-3000	_	2929.87	_
10.	C=Ostretch (aldehyde,saturated aliphatic)	1720-1740	_	1734.01	_
11.	C-Nstretch (aliphatic amines)	1020-1250	_	1215.15	1230.15
12.	C-Nstretch (aliphatic amines)	1020-1250	_	1053.13	1072.15
13.	O-Hbending (carboxylic acid)	950-910	_	927.76	928.17
14.	C-Br stretch (alkylhalides)	690-515	_	667.37	_
15.	CΞNstretch (alkynes)	2260-2100	_	_	2223.92
16.	C=Cstretch (alkenes)	1900-2000	_	_	2061.90

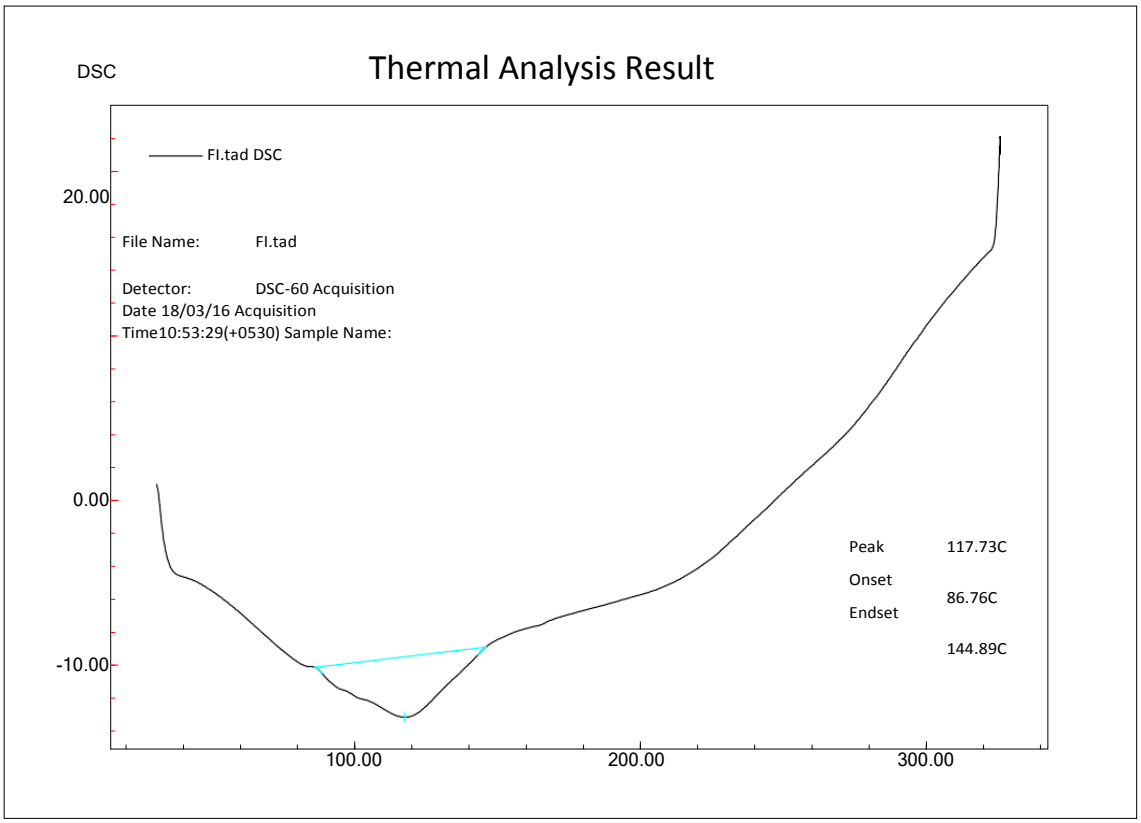

Fig. 7. DSC thermogram of *Momordica charantia* aqueous extract

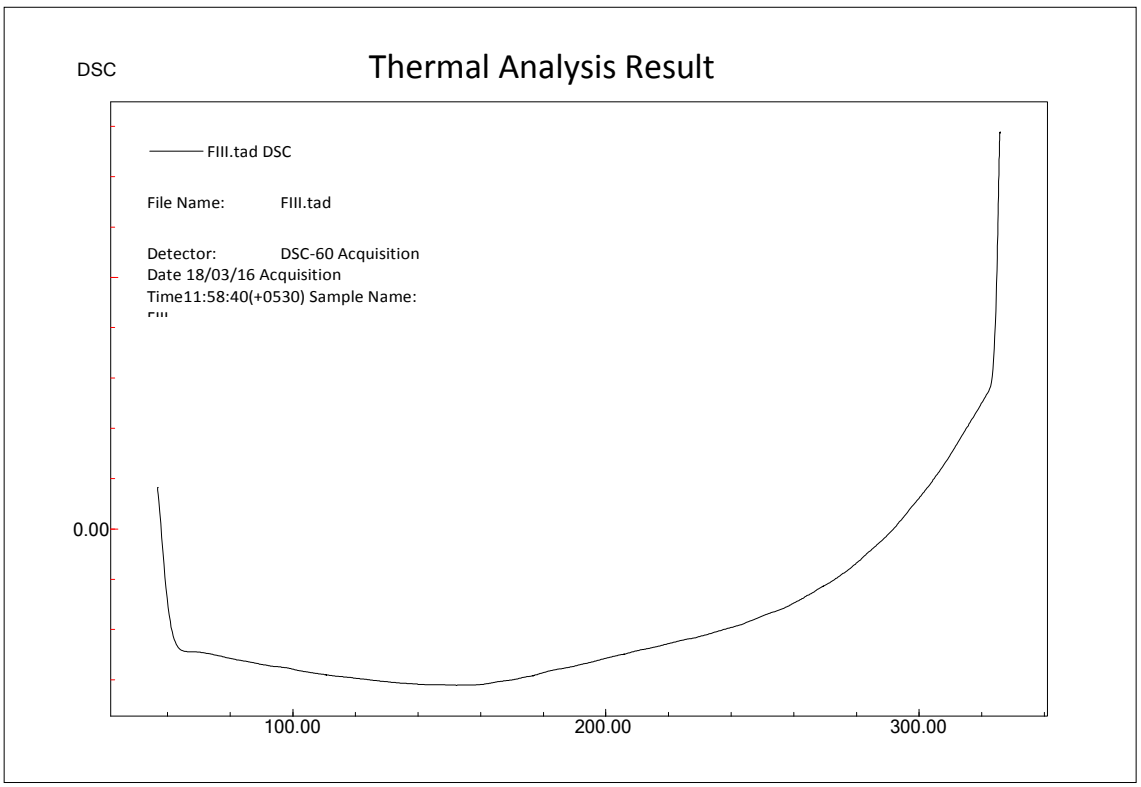

Fig. 8. DSC thermogram of phosphotidyl choline and cholesterol

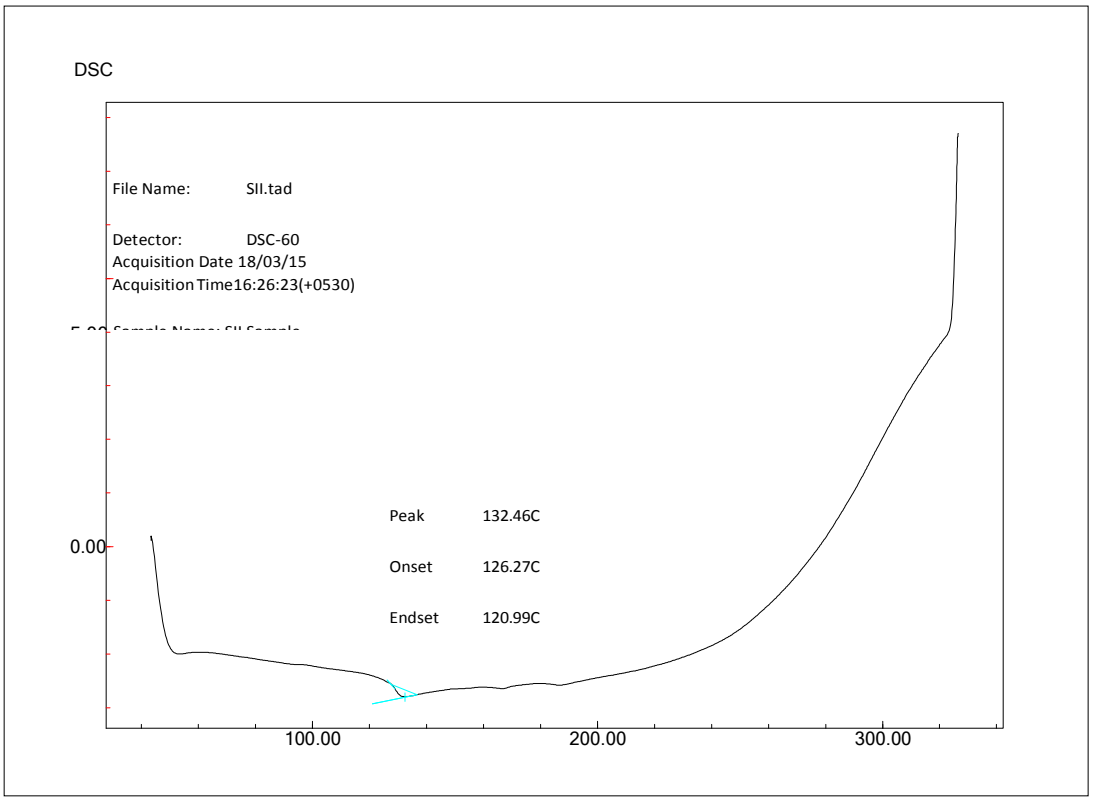

Fig. 9. DSC thermogram of MCAE with excipients

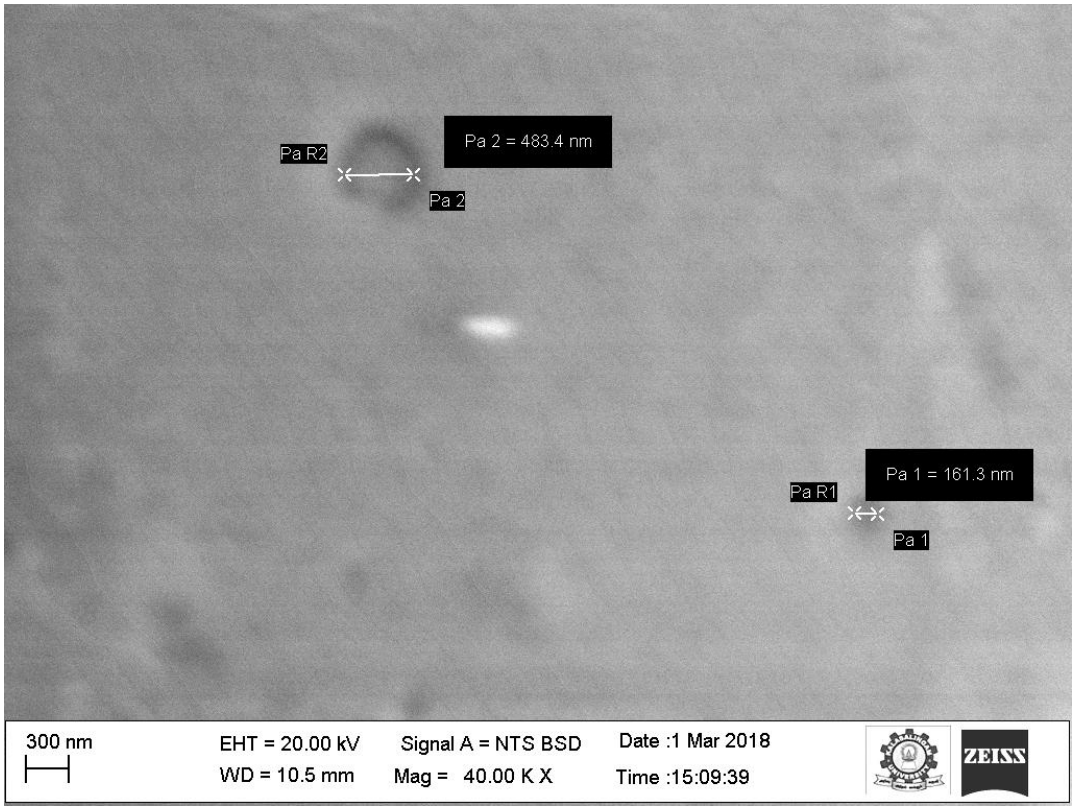

Fig. 10. SEM images for aqueous extract of *Momordica charantia* nanophytosomes

3.4 Differential Scanning Calorimetry (DSC) Studies [38-40]

Differential scanning calorimetry studied were conducted the pure MCAE, cholesterol, phosphatidylcholine. The endothermic peak of MCAE was observed at 117.7 ºC (Fig. 6) corresponding to its melting point. DSC thermogram of phosphotidyl choline and cholesterol also showed endothermic peaks at 159.4°C, respectively (Fig. 6). DSC Thermogram of MCAE with excipients (Fig. 7) showed endothermic peaks 132.46^0C. When compared the endothermic peak of extract (117.7^0C) with endothermic peak of extract placebo (132.46^0C) showed that there is no wide variation between the endothermic peaks and the difference is within ($\pm 20^0$C). This slight variation in the endothermic peaks may be due to the physical interaction between extract and phenol group OH.

3.5 Scanning Electron Microscopy Studies [41-43]

Scanning electron microscopy give important insight into the solid state properties and surface morphology of drug and drug complexes. SEM images (Fig. 8) of prepared MCAE nanophytosomes, respectively. These images showed spherical shaped MCAE nanophytosomes with a size of 100-500nm.

4. CONCLUSION

Nanophytosomes of aqueous extract of *Momordica charantia* was successfully prepared and tested for breast cancer cell lines. The aqueous extract was evaluated phyto chemical screening followed by all characterization studies. Phytochemical screening study remaining that the extract consists of flavanoids. The characterization study showed that the phytosomes are having nano size, good stability properties with round to spherical shape with smooth surfaces.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Keerthi B, Pingali PS, Srinivas P. Formulation and evaluation of phytosomes. International Journal of Pharmaceutical Science and Research. 2014;29:138-142.
2. Amin T, Bhat SV. A review on phytosome technology as a novel approach to improve the bioavailability of nutraceuticals. International Journal of Advanced Research and Technology, 2012;1:1-15.
3. Singh J, Khanra P, Kuila T, Srivastava M, Das AK, Kim NH, Jung BJ, Lee SH, Lee DW, Kim DG, Lee JH. Preparation of sulfonated poly (ether–ether– ketone) functionalized ternary for efficient glucose biosensor, Process Biochemistry. 2013;48:1724-35.
4. Kidd PM, Head K. A review of the bioavailability and clinical efficacy of milk thistle Phytosome: a silybin phosphatidylcholine complex. Alternative Medicine Review. 2005;10:193-203.
5. Semalty A, Semalty M, Rawat MSM. The phyto-phospholipid complexes-phytosomes: a potential therapeutic approach for herbal hepatoprotective drug delivery. Pharmacognosy Reviews. 2007;12:369-374.
6. Naik SR, Panda VS. Hepatoprotective effect of Ginkgo select Phytosome in rifampicin induced liver injury in rats: evidence of antioxidant activity. Fitoterapia. 2008;78:439-445.
7. Karimi N, Ghanbarzadeh B, Hamishehkar H, Pezeshki A, Mostafayi H, Gholian M. Phytosome as novel delivery system for nutraceutical materials. International Journal of Current Microbiological Applications and Science. 2015;4:152-159.
8. Rani B, Vandana NM, Nagpal M, Arora S. Phytosomes: potential carriers for herbal drugs. International Journal of Pharma and Applied Sciences, 2007;(2):566-577.
9. Pandey S, Patel K . A recent review on phytosomes. International Journal of Pharma Research, 2010;(2):627-631.
10. Saha S, Sarma K, Saikia P, Chakrabarty T. Phytosomes A brief overview, Scholars Academic Journal of Pharmacy. 2013;(2): 12-20.

11. Patel A, Tanwar Y, Rakesh S , Patel P. Phytosome: Phytolipid DrugDelivery System for Improving Bioavailability of Herbal Drug. Journal of Pharmaceutical Science and Bio scientific Research, 2013;(3):51-57.
12. Kumari P, Singh N, Cheriyan P, Neelam S. Phytosome: a novel approach for phytomedicine. International Journal of Institutional Pharmacy and Life Sciences, 2011;(1);89-100.
13. Patel J, Patel R, Khambholja K, Patel N. An overview of phytosomes as an advanced herbal drug delivery system. Asian Journal of Pharmaceutical Sciences, 2009;(4):363-371.
14. Pawar HA, Bhangale BD. Phytosome as a novel biomedicine: a microencapsulated drug delivery system. Journal of Bioanalysis & Biomedicine,2015;(7):6-10
15. Tripathy S, Patel D, Baro L, Nair S. A review on phytosomes, their characterization, advancement and potential for transdermal application. Journal of Drug Delivery and Therapeutics, 2013(3):147-152.
16. Gupta NK, Dixit VX. Development and evaluation of vesicular system for curcumin delivery. Archieves of Dermatological Research,2011;(303)89-101.
17. Soomro, A.K. , Ansari ,K.A. Medicinal uses of Bitter gourd (Momrdica charantia), Hamdard Med 2005;(48):9-14.
18. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia* : a review. Journal of ethnopharmacology. 2004;93(1):123-32.
19. Basch E, Gabardi S, Ulbricht C. Bitter melon (*Momordica charantia*): a review of efficacy and safety. American Journal of Health-System Pharmacy. 2003;(4):356-9.
20. Ali MM, Borai IH, Ghanem HM, Abdel-Halim AH, Mousa FM. The prophylactic and therapeutic effects of *Momordica charantia* methanol extract through controlling different hallmarks of the hepatocarcinogenesis. Biomedicine & Pharmacotherapy. 2018;(98):491-8.
21. Sur S, Steele R, Aurora R, Varvares M, Schwetye KE, Ray RB. Bitter Melon Prevents the Development of 4-NQO–Induced Oral Squamous Cell Carcinoma in an Immunocompetent Mouse Model by Modulating Immune Signaling. Cancer Prevention Research. 2018;11(4):191-202.
22. Molling JW, Moreno M, van der Vliet HJ, van den Eertwegh AJ, Scheper RJ, von Blomberg BM, Bontkes HJ. Invariant natural killer T cells and immunotherapy of cancer. Clinical Immunology. 2008;129(2):182-94.
23. Dia VP, Krishnan HB. BG-4, a novel anticancer peptide from bitter gourd (*Momordica charantia*), promotes apoptosis in human colon cancer cells. Scientific reports. 2016;6:33532.
24. Alshehri MA. Anticancer activity of methanolic extarct of *Momordica charantia* against human colon, liver and breast cancer cell lines-In vitro. Cell. 2016;6(6).
25. Yung MM, Ross FA, Hardie DG, Leung TH, Zhan J, Ngan HY, Chan DW. Bitter melon (*Momordica charantia*) extract inhibits tumorigenicity and overcomes cisplatin-resistance in ovarian cancer cells through targeting AMPK signaling cascade. Integrative cancer therapies. 2016;15(3):376- 89.
26. Shobha CR, Vishwanath P, Suma MN, Prashant A, Rangaswamy C, Gowdappa BH. In vitro anti-cancer activity of ethanolic extract of *Momordica charantia* on cervical and breast cancer cell lines. International Journal of Health & Allied Sciences. 2015;4(4):210.
27. Manoharan G, Cummings E, Singh J. Effects of crude water-soluble extract of *Momordica charantia* on viability, caspase activity, cytochrome-c release and on cytosolic calcium levels in different cancer cell lines. Cancer Cell & Microenvironment. 2014;1(3).
28. Abozaid SA, Baraka HN, Ibrahim AS, Gohar AA, Badria FA. Journal of Drug Discovery and Therapeutics 2 (13) 2014, 60-65. Journal of Drug Discovery and Therapeutics. 2014;2(13):60-5.
29. Li CJ, Tsang SF, Tsai CH, Tsai HY, Chyuan JH, Hsu HY. *Momordica charantia* extract induces apoptosis in human cancer cells through caspase- and mitochondria-dependent pathways. Evidence-Based Complementary and Alternative Medicine. 2012;2012.
30. Pitchakarn P, Suzuki S, Ogawa K, Pompimon W, Takahashi S, Asamoto M, Limtrakul P, Shirai T. Kuguacin J, a triterpeniod from *Momordica charantia* leaf, modulates the progression of androgen-independent human prostate cancer cell line, PC3. Food and chemical toxicology. 2012;50(3-4):840- 7.
31. Ru P, Steele R, Nerurkar PV, Phillips N, Ray RB. Bitter melon extract impairs

prostate cancer cell-cycle progression and delays prostatic intraepithelial neoplasia in TRAMP model. Cancer Prevention Research. 2011;4(12):2122-30.

32. Pitchakarn P, Suzuki S, Ogawa K, Pompimon W, Takahashi S, Asamoto M, Limtrakul P, Shirai T. Induction of G1 arrest and apoptosis in androgen-dependent human prostate cancer by Kuguacin J, a triterpenoid from *Momordica charantia* leaf. Cancer letters. 2011;306(2):142-50.
33. Brennan VC, Wang CM, Yang WH. Bitter melon (*Momordica charantia*) extract suppresses adrenocortical cancer cell proliferation and growth through modulation of the cell cycle pathway and steroidogenic activity.
34. Ray RB, Raychoudhuri A, Steele R, Nerurkar P. Bitter melon (*Momordica charantia*) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. Cancer Research. 2010 Mar 1;70(5):1925-31.
35. Pitchakarn P, Ogawa K, Suzuki S, Takahashi S, Asamoto M, Chewonarin T, Limtrakul P, Shirai T. *Momordica charantia* leaf extract suppresses rat prostate cancer progression in vitro and in vivo. Cancer science. 2010;101(10):2234-40.
36. Li M, Chen Y, Liu Z, Shen F, Bian X, Meng Y. Anti-tumor activity and immunological modification of ribosome-inactivating protein (RIP) from *Momordica charantia* by covalent attachment of polyethylene glycol. Acta Biochim Biophys Sin. 2009;(9):792-9.
37. Kobori M, Ohnishi-Kameyama M, Akimoto Y, Yukizaki C, Yoshida M. α- Eleostearic acid and its dihydroxy derivative are major apoptosis-inducing components of bitter gourd. Journal of Agricultural and food chemistry. 2008;(22):10515-20.
38. Beneveniste P, Hirth L, Oiurisson G. La biosyntheses sterol dans les tissue de tobacco cultiven in vitro ll. Particularity de la biosyntheses des phytosterols des tissue detabac cultivates in vitro. Phytochemistry.1966;(5):45- 68.
39. Budrat P, Shotipruk A. Extraction of phenolic compounds from fruits of bitter melon (*Momordica charantia*) with subcritical water extraction and antioxidant activities of these extracts. Chiang Mai J Sci. 2008;(1):123-30.
40. Zahra Hooresfand, Saeed Ghanbarzadeh, Hamed Hamishehkar. Preparation and characterization of rutin-loaded nanophytosomes. Pharmaceutical Sciences. 2015(1):145-151.
41. Demir B, Barlas FB, Guler E, Gumus PZ, Can M, Yavuz M, Coskunol H, Timur S. Gold nanoparticle loaded phytosomal systems: synthesis, characterization and in vitro investigations. RSC Advances. 2014;(65):34687- 95.
42. Zheng D, Wang Y, Zhang D, Liu Z, Duan C, Jia L, Wang F, Liu Y, Liu G, Hao L, Zhang Q. In vitro antitumor activity of silybin nanosuspension in PC-3 cells. Cancer letters. 2011;(2):158-64.
43. Belloc F, Dumain P, Boisseau MR, Jalloustre C, Reiffers J, Bernard P, Lacombe F. A flow cytometric method using Hoechst 33342 and propidium iodide for simultaneous cell cycle analysis and apoptosis determination in unfixed cells. Cytometry Part A. 1994;(1):59-65.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77932