

**PREPARATION AND EVALUATION OF NANOPHYTOSOMES OF AQUEOUS
EXTRACT OF LEAVES OF *MOMORDICA CHARANTIA***

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. *Momordica 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 *momordica 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.

Key words: Flavanoids, Momordica charantia, Nanophytosomes, Phytochemical screening,

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 an unidentified alkaloid and 5-hydroxytryptamine is also reported. The 5HT content is reported to be present. [4-6] The ether 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 an 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 melon seeds can be potentially used to destroy cancer cells and they were successfully administered to patients

inK andy 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]

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 hydrophilic (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]

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 is 1:1. 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]

Materials and Methods:

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.

Methodology

Collection and Processing of Momordica Charantia Plant Material

Momordica 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.

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 desicator at room temperature.

Preliminary Phytochemical Analysis[22-25]

The aqueous extract of momordica 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 μ L Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature ($28\pm 2^{\circ}\text{C}$), 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 $\mu\text{g/mL}$) was used as standard (Vijayalakshmi, Ravichandiran, Malarkodi, Nirmala, & Jayakumari, 2012).

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 ($45\text{-}50^{\circ}\text{C}$). 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 ($37\text{-}40^{\circ}\text{C}$ 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 ($2\text{-}8^{\circ}\text{C}$) 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.

Characterization of Nanophytosomes

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.

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 °C and 149 watt. The average three zeta potential determinations of the nanophytosomes were premeditated.

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.

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.

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 vacuum 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.

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.

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.

Results and Discussion

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]

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. H ₂ SO ₄	+
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. Bortrager'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

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 altitude of phytoconstituents which may possibly take part in reactions in effective reduction of nanophytosomes. (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]

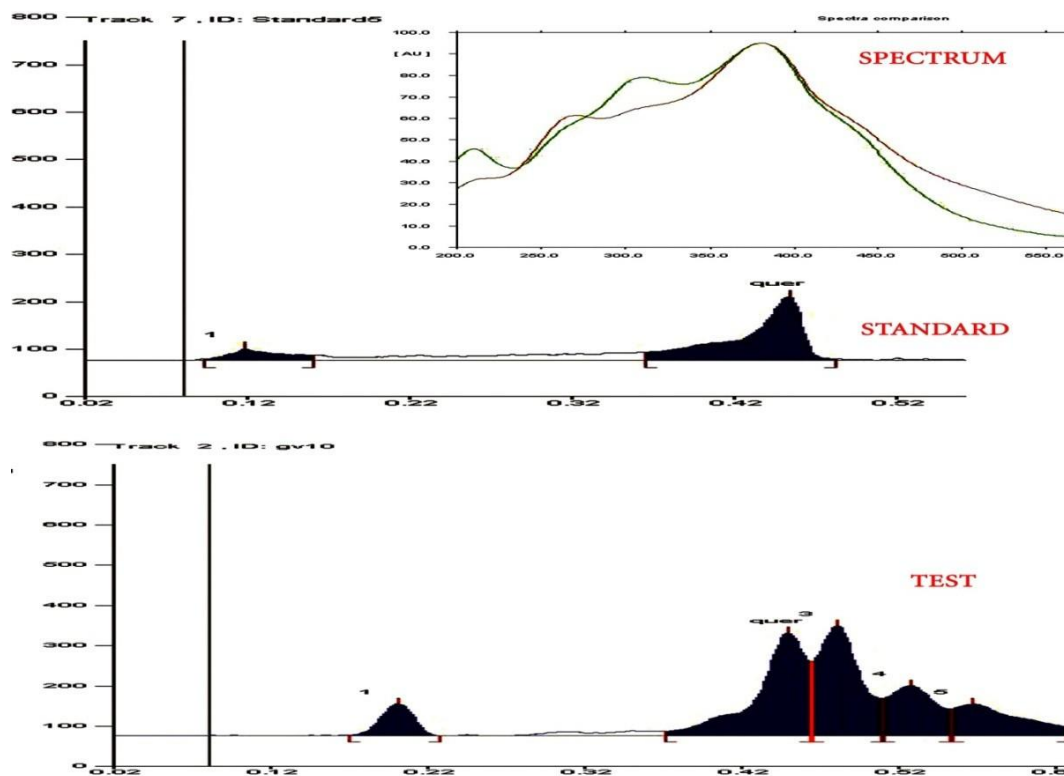


Figure-1: HPTLC finger printing analysis of aqueous extract of *momordica charantia* leaves

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 heterogeneity of sizes of particles in a mixture *momordica charantia* nanophytosomes prepared show polydispersity index value of 0.4. [33-35]

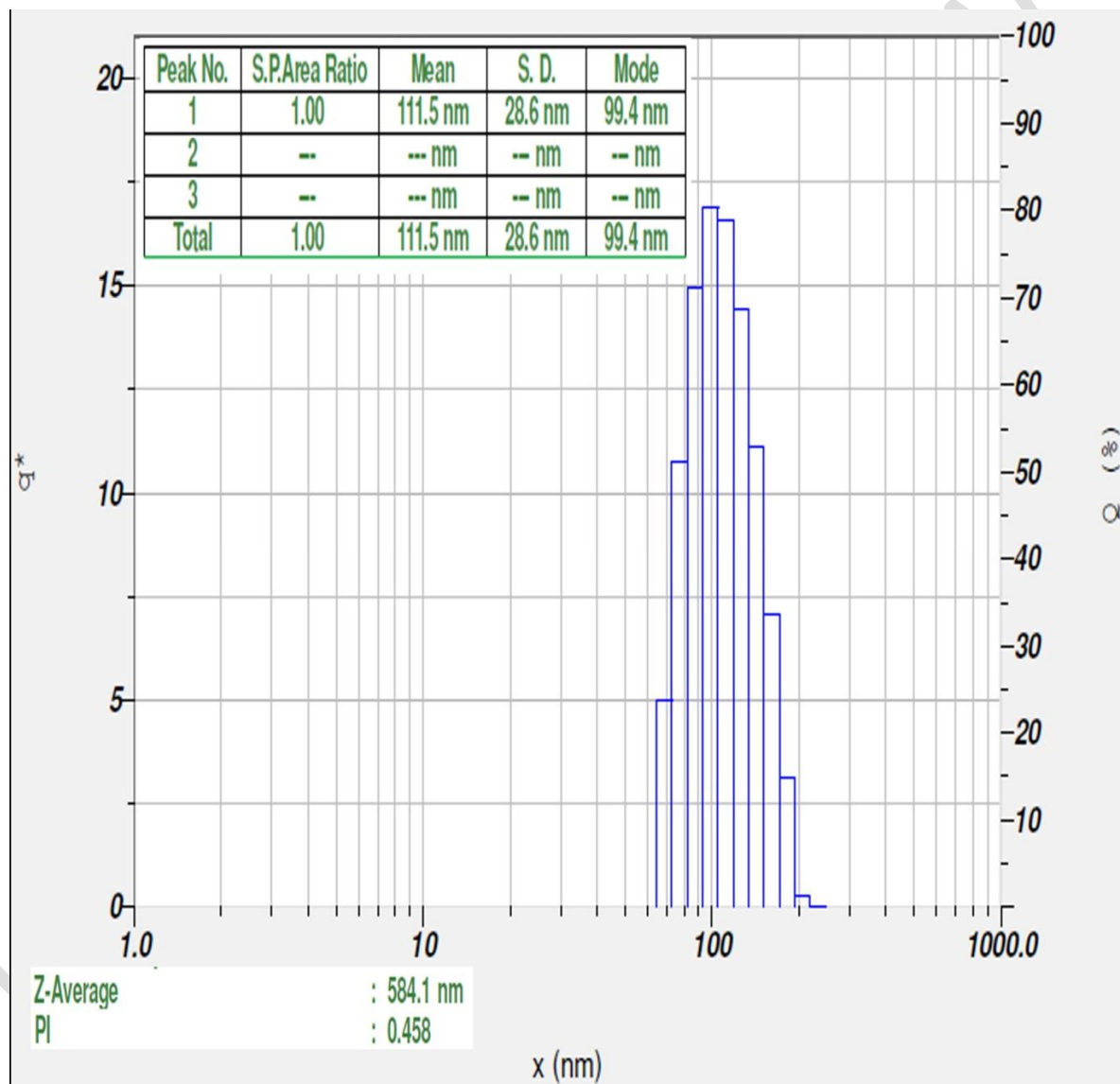
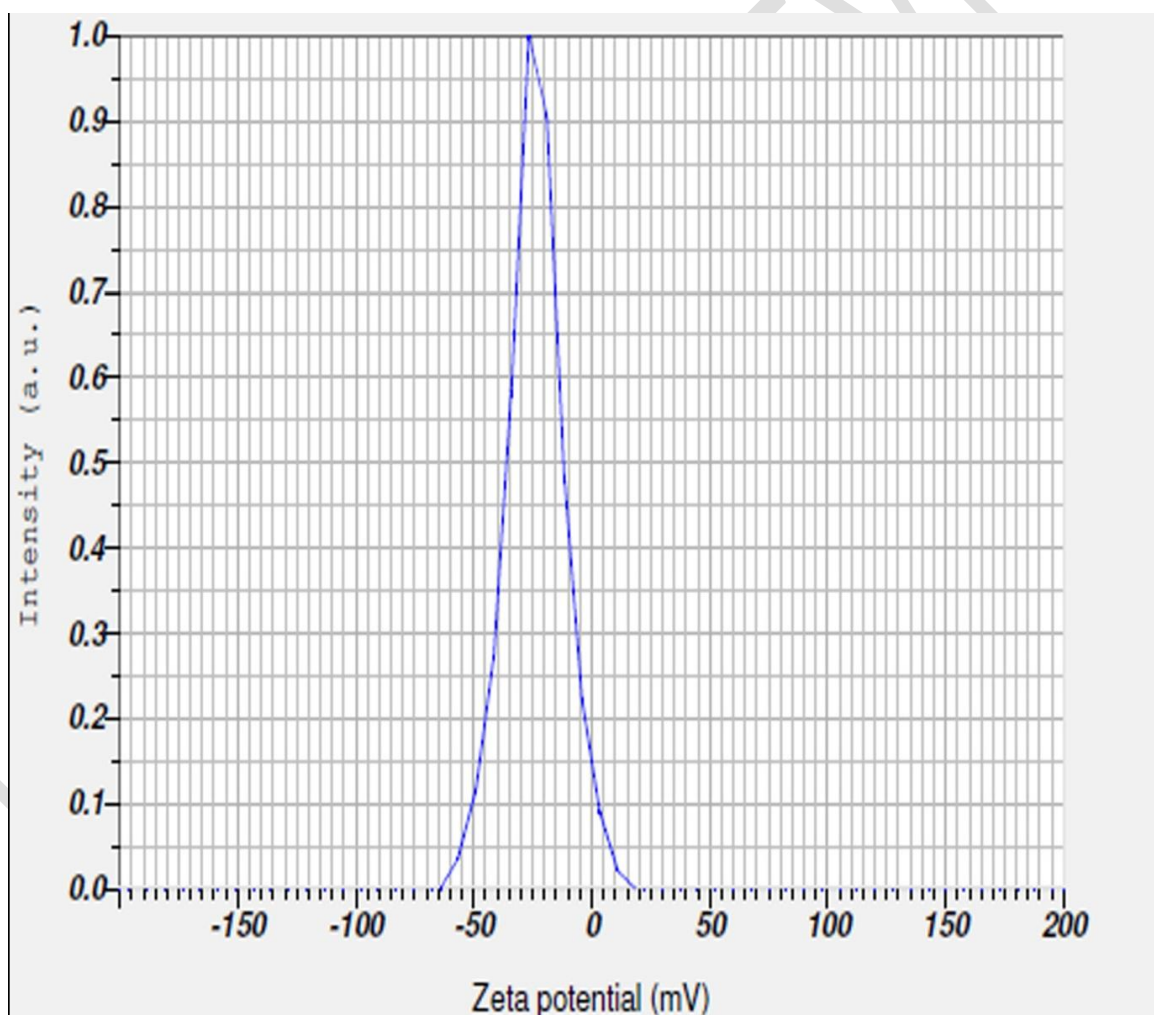


Figure-2 particle size of aqueous extract *momordica charantia* nanophytosomes

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]

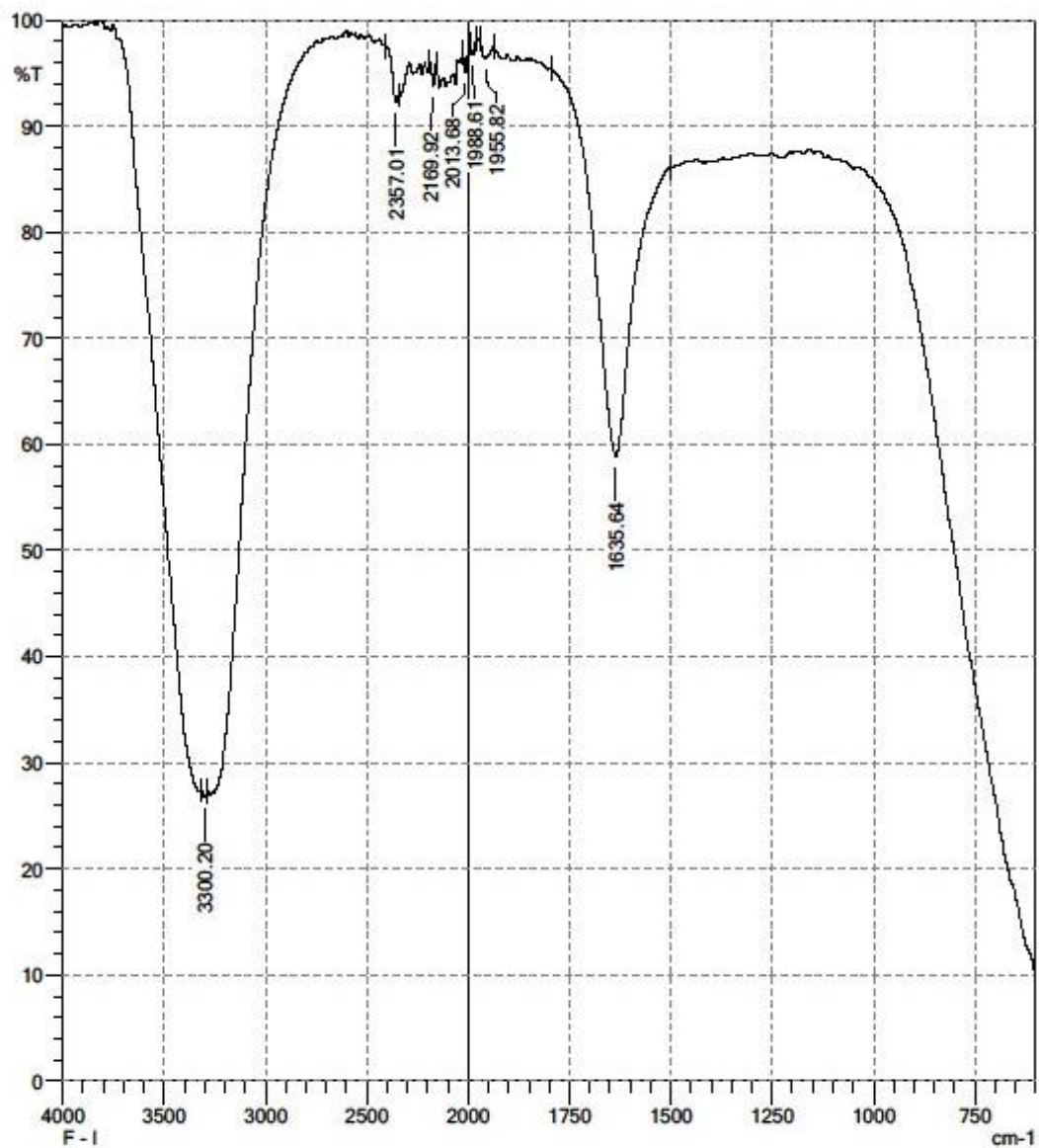


Zeta Potential (Mean) : -23.8 mV
Electrophoretic Mobility mean : -0.000184 cm²/Vs

Figure-3: Zeta Potential Distribution

Fourier Transform-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 Figure 4, 5, 6. (Figure-4) shows that the characteristic O-H peak at 3300.20cm^{-1} , $\text{C}\equiv\text{N}$ peak at 2357.01 cm^{-1} , $\text{C}\equiv\text{C}$ peak at 2169.92 cm^{-1} , $\text{C}=\text{C}$ peak at 1988.61 cm^{-1} , $\text{C}=\text{C}$ peak at 1635.64 cm^{-1} . (Figure 6.5) shows that the C-H peak at 3020.53 cm^{-1} , $\text{C}=\text{C}$ peak at 1988.61 cm^{-1} , $\text{C}=\text{O}$ 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} . (Figure 6) shows that the characteristic O-H peak at 3302.13 cm^{-1} , $\text{C}\equiv\text{N}$ peak at 2360.87 cm^{-1} , $\text{C}\equiv\text{C}$ peak at 2167.99cm^{-1} , $\text{C}=\text{C}$ peak at 1990.54 cm^{-1} , $\text{C}=\text{C}$ peak at 1635.64 cm^{-1} . Hence there is no appearance of new peaks and disappearance of existence peaks in the presence of excipients indicates the MCAE and excipients are more compatible. [37]

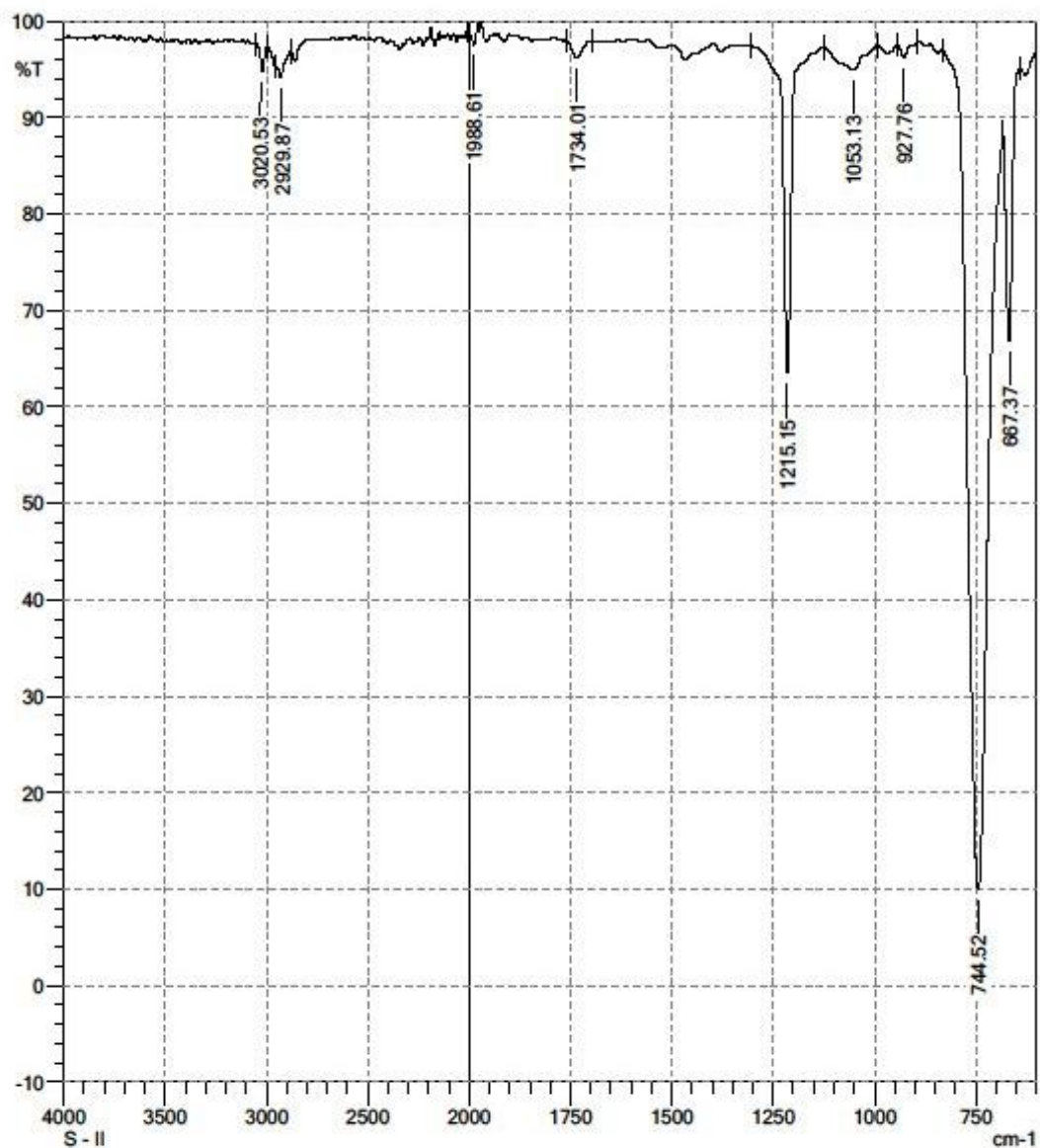


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Figure-4: FT-IR studies of *momordica charantia* aqueous extract

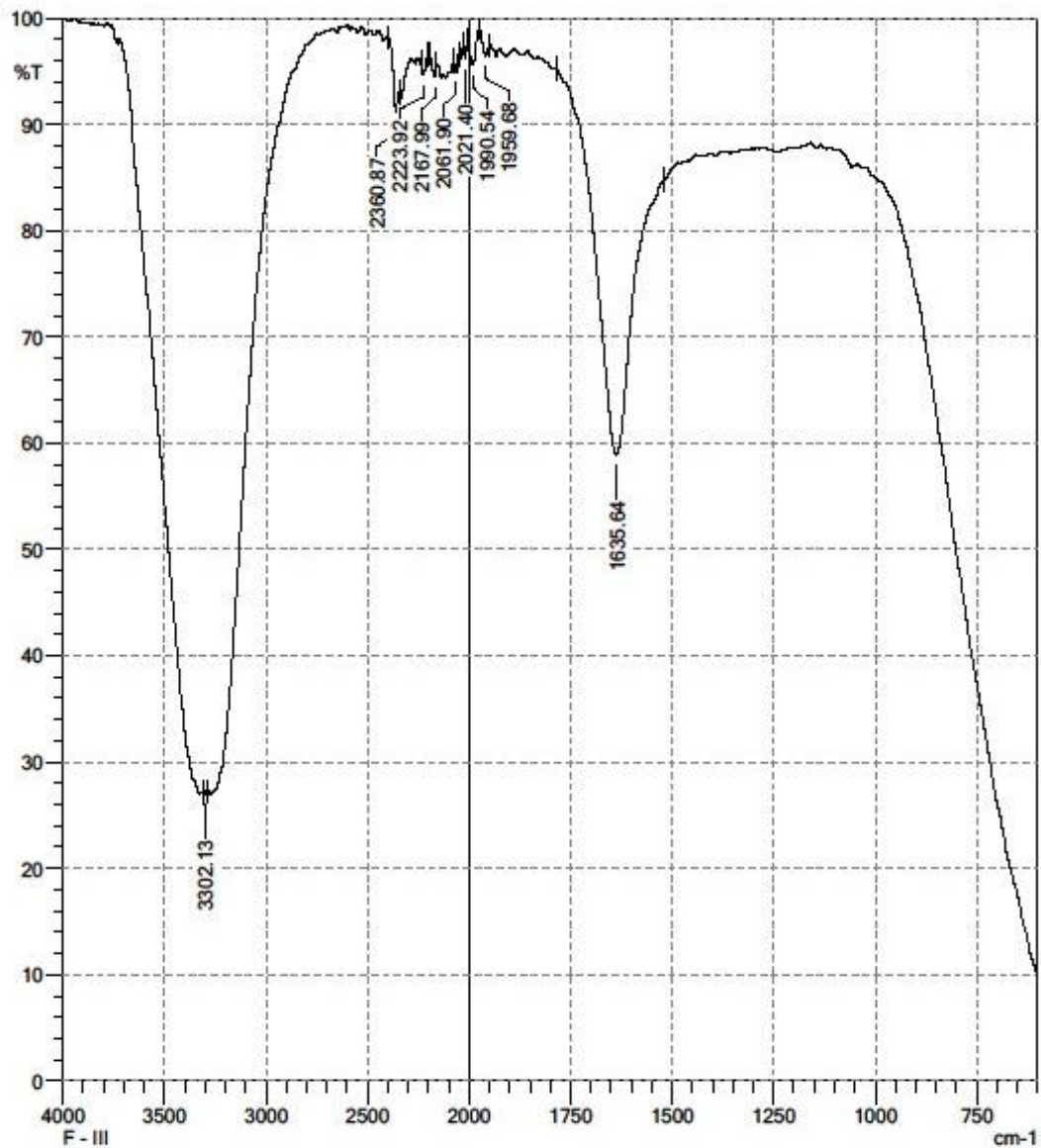


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Figure-5: FT-IR studies of phosphodityl-choline with cholesterol.



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Figure-6: FT-IR studies of *momordica charantia* aqueous extract with excipients.

Table-2: Interpretation of FT-IR studies

S.No.	Functional group	Wavenumber(cm^{-1})			
		Reference	Extract	Placebo	Extract+placebo
1.	O-H Stretch(carboxylic acid)	3300-2500	3300.20	–	3302.13
2.	$\text{C}\equiv\text{N}$ stretch(nitriles)	2260-2210	2357.01	–	2360.87
3.	$\text{C}\equiv\text{N}$ stretch(alkynes)	2260-2100	2169.92	–	2169.99
4.	$\text{C}=\text{C}$ stretch(alkenes)	1900-2000	2013.68	1988.61	2021.40
5.	$\text{C}=\text{C}$ stretch(alkenes)	1900-2000	1988.61	–	1990.54
6.	$\text{C}=\text{C}$ stretch(alkenes)	1900-2000	1955.82	–	1959.68
7.	$\text{C}=\text{C}$ stretch(alkenes)	1640-1680	1635.64	–	1635.64
8.	C-H stretch(aromatic)	3100-3000	–	3020.53	–
9.	C-H stretch(alkenes)	3100-3000	–	2929.87	–
10.	$\text{C}=\text{O}$ stretch(aldehyde, saturated aliphatic)	1720-1740	–	1734.01	–
11.	C-N stretch(aliphatic amines)	1020-1250	–	1215.15	1230.15
12.	C-N stretch(aliphatic amines)	1020-1250	–	1053.13	1072.15
13.	O-H bending(carboxylic acid)	950-910	–	927.76	928.17
14.	C-Br stretch(alkyl halides)	690-515	–	667.37	–
15.	$\text{C}\equiv\text{N}$ stretch(alkynes)	2260-2100	–	–	2223.92
16.	$\text{C}=\text{C}$ stretch(alkenes)	1900-2000	–	–	2061.90

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

Differential scanning calorimetry studies were conducted on pure MCAE, cholesterol, phosphatidylcholine. The endothermic peak of MCAE was observed at 117.7 °C (Figure 6.5) corresponding to its melting point. DSC thermogram of phosphatidyl choline and cholesterol also showed endothermic peaks at 159.4°C, respectively (Figure-6). DSC Thermogram of MCAE with excipients (Figure 6.7) showed endothermic peaks at 132.46°C. When compared the endothermic peak of extract (117.7°C) with endothermic peak of extract placebo (132.46°C) showed that there is no wide variation between the endothermic peaks and the difference is within ($\pm 20^{\circ}\text{C}$). This slight variation in the endothermic peaks may be due to the physical interaction between extract and phenol group OH.

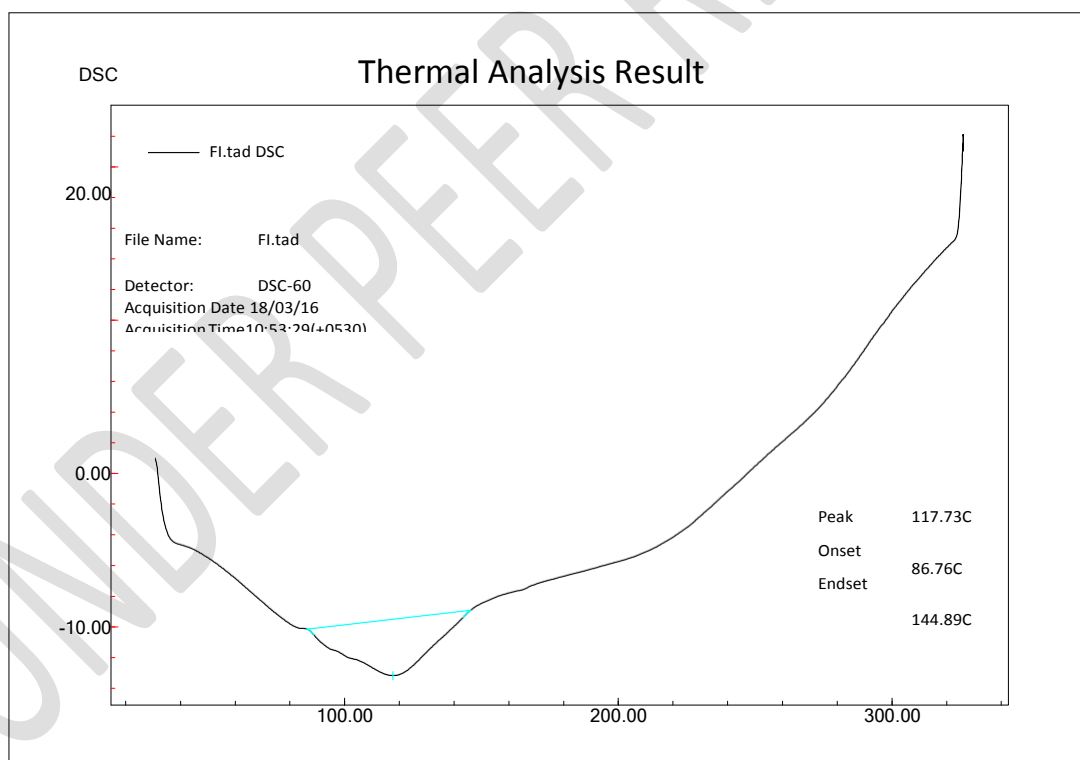


Figure-7: DSC thermogram of *momordica charantia* aqueous extract.

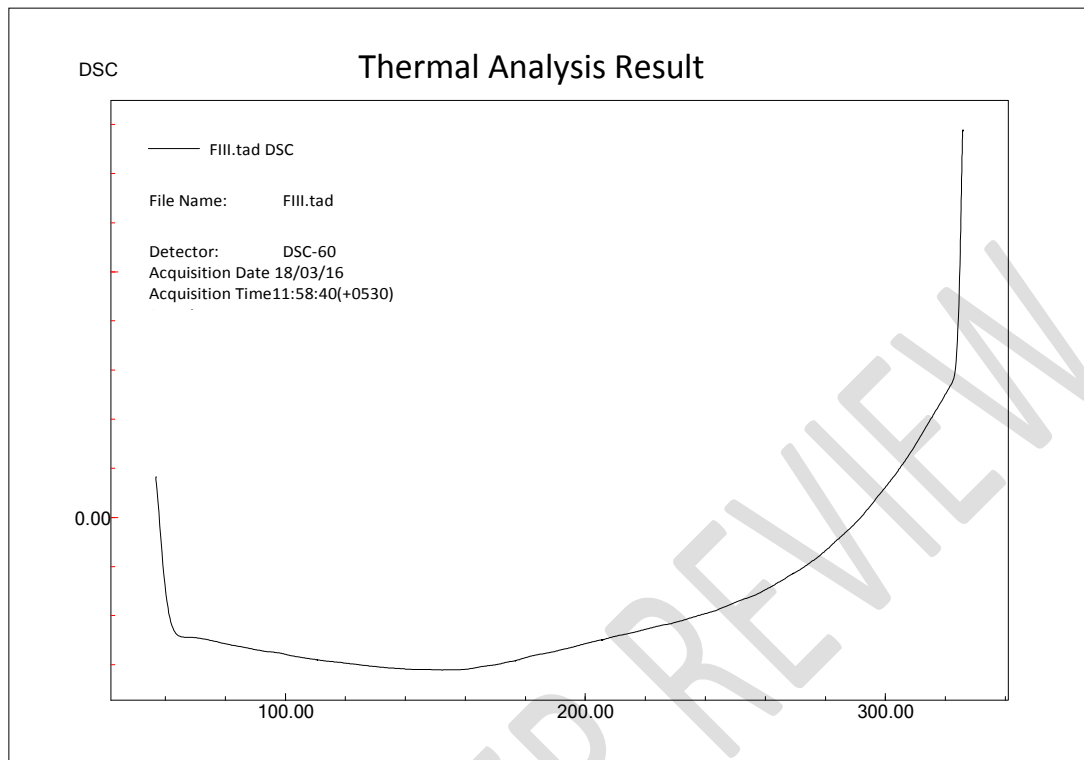


Figure-8: DSC thermogram of phosphatidyl choline and cholesterol.

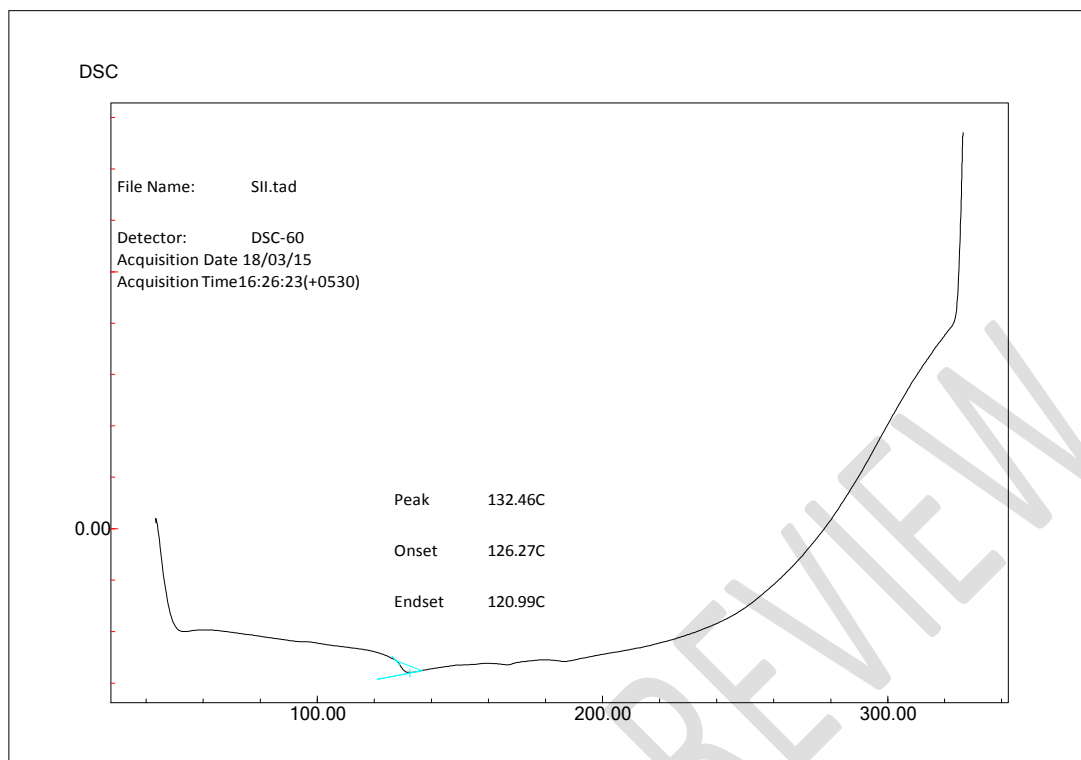
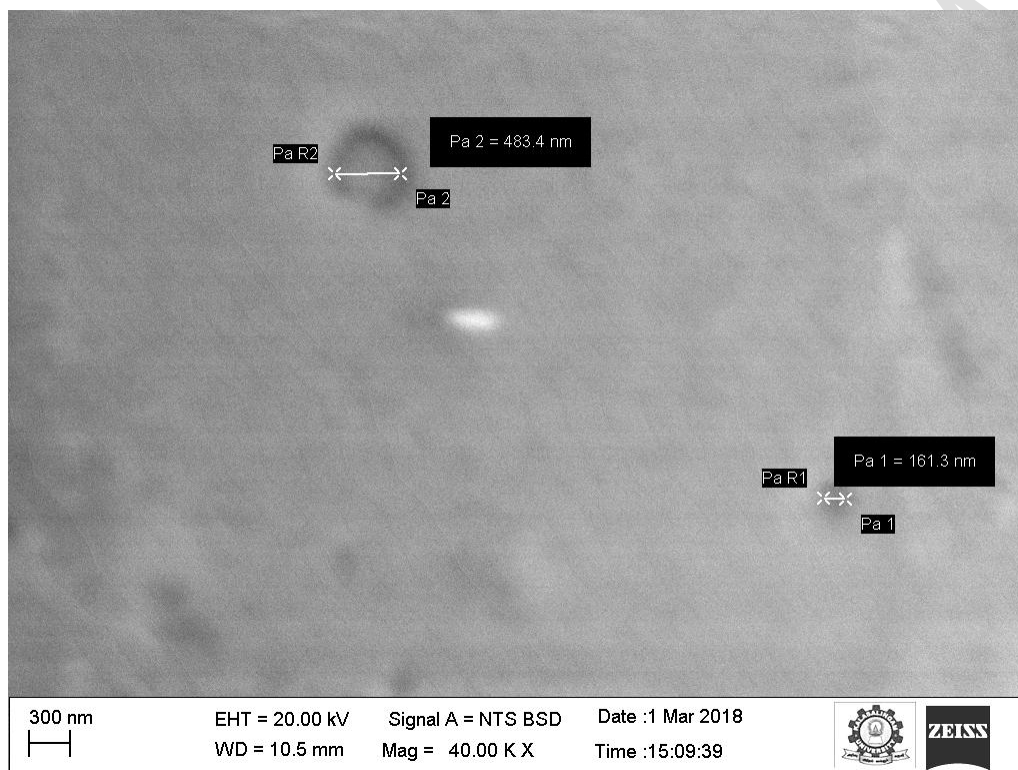


Figure-9: DSC thermogram of MCAE with excipients.

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(Figure-8) of prepared



MCAE nanophytosomes ,respectively. These images showed spherical shaped MCAE nanophytosomes with a size of 100-500nm.

Figure-10: SEM images for aqueous extract of *momordica charantia* nanophytosomes

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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