https://ijrps.com/

ISSN: 0975-7538
Research Article

Compritol Solid Lipid Microparticles Loaded with Herbal Extracts for Acne Treatment

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ABSTRACT

Acne Vulgaris is an inflammation of sebaceous glands characterized by pustules and skin lesions. The usage of herbal cosmetics has been increased to many folds in personal care system and there is a great demand for the herbal cosmetics. The use of bioactive ingredients in cosmetics influence biological functions of skin and provide nutrients necessary for the healthy skin or hair. The present research work deals with Development and evaluation of solid lipid micro particles loaded with Grapes (*Vitis venifera*) seed and Marigold (*Calendula officinalis*) flower extract, and essential oils of Sea buckthorn (*Hippophae rhamnoides*)seeds for treatment of acne vulgaris. Compritol (5.0% wt/wt) SLM dispersions were prepared by oil in water emulsification method, using different surfactant concentrations and Extract concentration. The SLM were characterized, in terms of surface morphology, particle size and stability. Solid lipid micro particle technology represents a promising new approach to lipophilic drug delivery. Herbal extract and oils were screened Phytochemical and TLC, HPLC were performed for Qualitative and Quantitative analysis of active constituents present. The formulation have been developed and evaluated,

Keywords: Acne Vulgaris; Inflammation; Solid Lipid Microparticles

INTRODUCTION

Acne vulgaris remains one of the most common diseases afflicting humanity and it is the skin disease most commonly treated by physicians. It is a disease of the pilosebaceous units, clinically characterized by seborrhea, comedones, papules, pustules, nodules and, in some cases, scarring (Adityan B et al 2009). Acne vulgaris is an inflammation of sebaceous glands characterized by pustules and skin lesions. Various bacteria, including chiefly P. acnes and S. epidermis, have been identified as the etiological agents of acne (Askenazi H.etal 2003,Higaki s. 2003).

Acne typically appears on your face, neck, chest, back and shoulders, which are the areas of your skin with the largest number of functional oil glands. Follicles, often called pores, sometimes get blocked. Sebum (oil) which normally drains to the surface gets blocked and bacteria begin to grow (Webster GF 2005).

In recent years, biocompatible lipid micro and nano particles have been reported as potential drug carrier systems as alternative materials to polymers. Micro particulate drug delivery system is one of the processes to provide the sustained & controlled delivery of drug to long periods of time (Madhav and Kala 2011).

Solid lipid particles have been proposed as a colloidal drug carrier therapeutic system for different administration routes such as oral, topical, ophthalmic, and subcutaneous and intramuscular injection, and particularly for parenteral administration (pulmonary). Solid lipid Microparticles are similar to conventional oil-in- water (o/w) emulsions; in which oil droplets, containing a hydrophobic API, are suspended in a polar solvent with a surfactant stabilizer. However, in comparison to conventional o/w emulsions, a higher molecular weight hydrophobic “oil” or saturated lipid component are used which is solid at room temperature. The solid lipid is melted, and the hydrophobic drug is dissolved into it. This melted solution is subsequently mixed into a polar phase and homogenized to form small o/w droplets that can then be cooled to form solid lipid Microparticles (Mezzena M.et al 2009).

Personal care industry is currently more concentrated on these herbal-based cosmetics as now- a-days it is a fast growing segment with a vast scope of manifold expansion in coming years. The use of bioactive ingredients in cosmetics influence biological functions of skin and provide nutrients necessary for the healthy skin or hair. In general, botanicals provide different vitamins, antioxidants, various oils, essential oils, hydrocolloids, proteins, terpenoids and other bioactive

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Received on: 25-01-2012
Revised on: 09-03-2012
Accepted on: 11-07-2012

Table 1: Formula for Solid Lipid Microparticles

SN	Formulation	Polymer (g)	Extract(g)			Surfactant (g)	Water (g)
			Calendula Extract	Vitis vinifer Extract	Sea buckthorn oil		
1	SLM1	5	2.4	1	1.9	0.6	qs
2	SLM2	5	2.4	1	1.9	0.4	qs
3	SLM3	5	2.4	1	1.9	0.2	qs
4	SLM4	5	2.89	1	0.6	0.2	qs
5	SLM5	5	2.4	1	2.1	0.2	qs
6	SLM6	5	2.4	2.5	0.6	0.2	qs

Table 2: Organoleptic properties

S.N.	Parameter	Description		
		Calendula	Vitis vinifer	Sea Buck Thorn
i)	Color	Yellow	Dark brown	Pale yellow
ii)	Odors	No odor	Wine	No odor

molecules. There is tremendous scope to launch numerous herbal cosmetics using appropriate bioactive ingredients with suitable fatty oil, essential oils, proteins and additives (Dr.Babu S.)

In this study we investigate potential of Microparticles having herbal extract for acne treatment. Extracts were prepared and subjected to Phytochemical screening, particles containing herbal extract were prepared using oil in water emulsification technique and studied for their size, morphology, drug loading capacity.

MATERIAL AND METHOD

Materials

Calendula officinalis, Grape seed extract, sea buckthorn oil were obtained from Amruta Herbals Pvt Ltd,Indore, Compritol 888(Glycerol Behnate),Pluronic F-68 .

Methods

Phytochemical screening and Characterization of Extracts Calendula officinalis, Grape seed extract and se buck thorn oil were subjected to Phytochemical screening. Various test for Alkaloids, Flavonoids, Carbohydrate, Proteins, Oils etc were performed and presence of Phytoconstituents (Khandelwal R.2008). Herbal extracts were then studied for their solubility, physical appearance.

HPLC assay

HPLC is performed for Calendula officinalis, Vitis Vinifer extract and sea buckthorn oil for quantification of Constituents. Method used for HPLC of Extracts was performed.

Calendula officinalis

The HPLC system consisted of a solvent delivery module LC-20AT, and a UV-Vis Spectrophotometeric detector (Model SPD-20A), all of which were obtained from Shimadzu Corporation, Tokyo, Japan. Column used was C-18, with the flow rate was 1ml/min and detection at 340 nm.

Mobile phase

Acetonitrile (15 ml) was mixed with Water (85 ml). The solution was agitated on a magnetic stirrer for 10-15 minutes to ensure uniform mixing, to this solution 1-2 drops of 2% acetic acid was added and mixing was continued. The solution was then filtered through a 0.22 µm filter prior to be used in HPLC.

Standard solutions

Standard solutions for Flavonoids were prepared in mobile phase. It was reconstituted with mobile phase to give a stock solution of 1000 mg/ml. various standard solutions were prepared from this stock solution after adequate dilutions with the mobile phase.

Sample solutions

Sample was dissolved in acetone to get conc. 100 µg/ml, and from this solution, sample solutions with different were prepared.

Calculations

The unknown Extract concentration was determined by interpolating from the regression equation relating to the peak area, obtained from the set of standard solutions.

Vitis vinifer extract

The HPLC system consisted of a solvent delivery module LC-20AT, and a UV-Vis Spectrophotometeric detector (Model SPD-20A), all of which were obtained from Shimadzu Corporation, Tokyo, Japan. Column used was C-18, with the flow rate was o.5 ml/min and detection at 254 nm.

Mobile phase

Methanol (40 ml) & Water (55ml) was mixed with Phosphoric acid (5 mL). The solution was agitated on a magnetic stirrer for 10-15 minutes to ensure uniform mixing; the solution was then filtered through a 0.22 µm filter prior to be used in HPLC. Standard solutions Standard solutions for Gallic acid were prepared in

Table 3: Solubility of Extracts

S.N.	Solvents	Calendula	Grape seed	Sea buck thorn
i)	Ethanol	+++	+++	+
ii)	Hexane	++	++	+++
iv)	Acetone	+	++	++
v)	Methanol	+++	+++	++
vi)	Chloroform	+++	++	++
vii)	Benzene	++	++	+++
viii)	Distilled water	+++	+++	-
ix)	PBS 7.4	+++	+++	-

[+very less soluble ++ less soluble +++Soluble]

Table 4: HPLC analysis of extracts

Extract	Flow Rate	Column	Mobile Phase	Detector	Wavelength
Calendula officinalis	1ml/min	C-18	Acetonitrile :Water	UV Visible	340
Grape seed	0.5ml/min	C-18	Methanol: Water	UV Visible	254
Sea buckthorn	1ml/min	C-18	Acetonitrile: Acetone	UV Visible	208

Table 5: Particle size determination of Microparticles

SN.	Formulation	Particle size(µm)± SD
1	SLM1	8.9 ± 1.6
2	SLM2	6.7 ± 1.1
3	SLM3	4.8 ±0.8
4	SLM4	4.5±0.2
5	SLM5	5.0±0.7
6	SLM6	4.8±1.3

Table 6: Drug loading and Encapsulation efficiency

SN	Formulation	Drug estimated	Drug added	% EE	% Loading
1	SLM 3	4.41gm	5.3gm	83.01%	44%
2	SLM 4	4.36	5.3	82.2%	43%
3	SLM 5	4.33	5.3	81.6%	43.2%
4	SLM 6	4.4	5.3	83.0%	43.5%

mobile phase. It was reconstituted standard solutions for Gallic acid was prepared in mobile phase. It was reconstituted with mobile phase to give a stock solution of 1000 mg/ml. various standard solutions were prepared from this stock solution after adequate dilutions with the mobile phase.

Sample solutions

Sample was dissolved in methanol to get conc. 100 µg/ml, and from this solution, sample solutions with different were prepared.

Calculations

The unknown Extract concentration was determined by interpolating from the regression equation relating to the peak area, obtained from the set of standard solutions.

***Sea buckthorn* oil**

The HPLC system consisted of a solvent delivery module LC-20AT, and a UV-Vis Spectrophotometeric detector (Model SPD-20A), all of which were obtained from Shimadzu Corporation, Tokyo, Japan. Column used was C-18, with the flow rate was 1ml/min and detection at 205 to 210 nm.

Mobile phase

Acetonitrile (90 ml) was mixed with acetone (10 ml). The solution was agitated on a magnetic stirrer for 10-15 minutes to ensure uniform mixing, to this solution 1-2 drops of acetic acid was added and mixing was continued. The solution was then filtered through a 0.22 µm filter prior to be used in HPLC.

Standard solutions

Standard solutions for fatty acid were prepared in mobile phase. It was reconstituted with mobile phase to give a stock solution of 1000 mg/ml. Various standard solutions were prepared from this stock solution after adequate dilutions with the mobile phase.

Sample solutions

Sample was dissolved in methanol to get conc. 100 µg/ml, and from this solution, sample solutions with different were prepared.

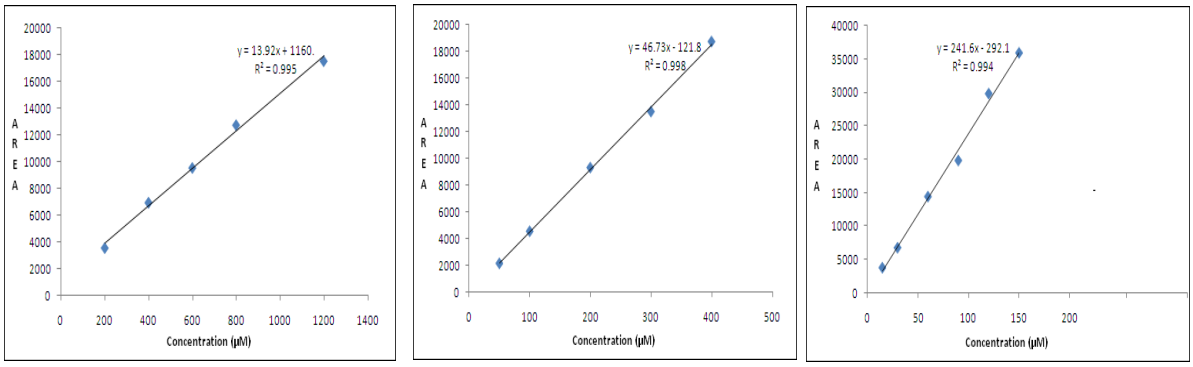

Figure 1: Calibration Curve of Calendula officinalis, Grape seed extract and Sea buckthorn oil

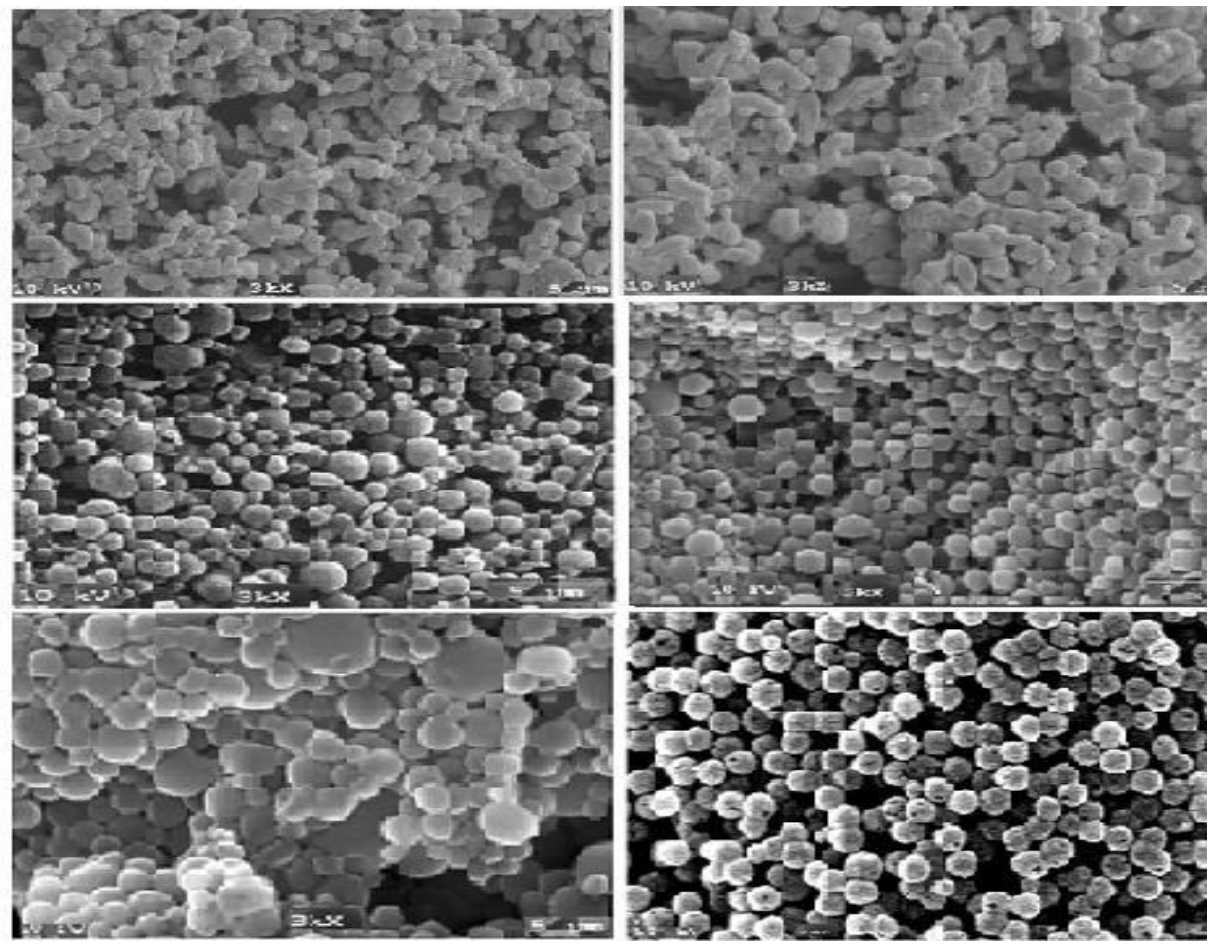

Figure 2: Scanning electron microscopy of Microparticles

Calculations

The unknown Oil concentration was determined by interpolating from the regression equation relating to the peak area, obtained from the set of standard solutions.[Christie W.W 2011, Dieneka V.I. et al 2009, Rodriguez D.et al2002, López M.et al 2010, Fuleki T,and Silva 1997]

Optimization and formulation of Solid Lipid Microparticles

SLM were obtained by oil in water (o/w) emulsification employing the phase inversion technique. Compritol, and the surfactant were heated to 90°C,Extract were added to it and hot purified water at the same temperature was slowly added to oily phase. The emulsions were prepared by Silverson, emulsifying at 6200 rpm. The emulsions obtained were cooled at room temperature under magnetic stirring until solidification of the Microparticles occurred. Optimization of the Microparticles was performed by Characterization of Microparticles and the Microparticles with good results were then subjected for further study (Mezzena M. et al 2009, Fonseca Y.M.et al 2009).

Evaluation of Microparticles

The prepared floating Microparticles were evaluated for various parameters such as particle size, X-Ray Diffraction, DSC, FTIR study, drug entrapment efficiency.

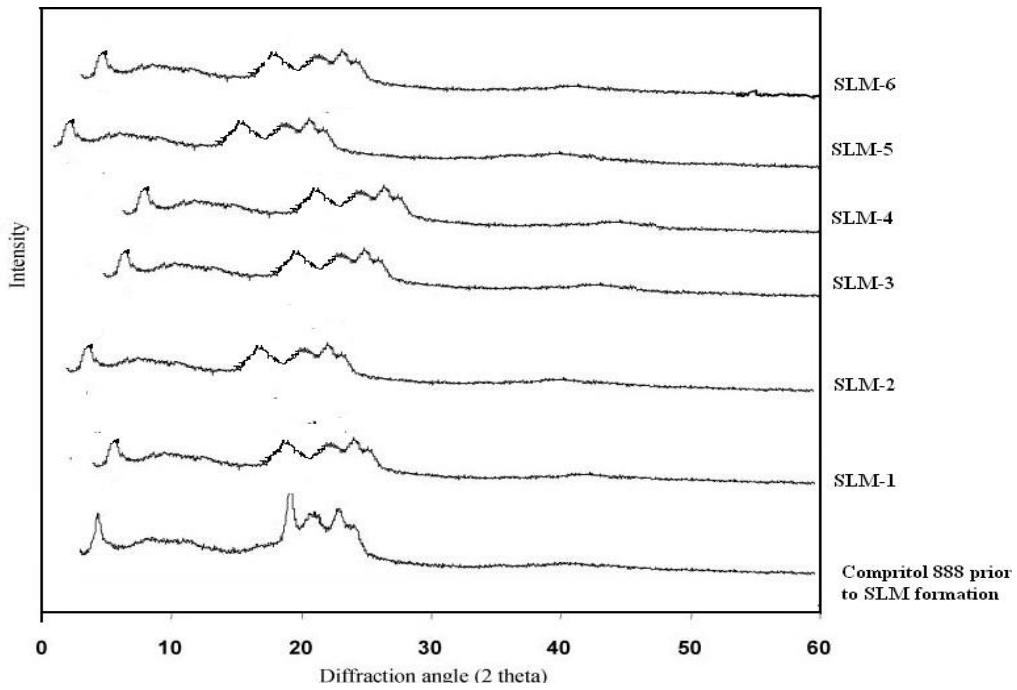

Figure 3: XRD images

Scanning Electron Microscopy

Microparticles samples were coated with gold/palladium under argon atmosphere and examined under a scanning electron microscope (SHIMADZU—SS550, Tokyo, Japan). The scanning electron photomicrographs (SEM) were analyzed (Avanço, G.B.& Bruschi 2008).

Particle size measurement

The particle size of the Microparticles was measured by optical microscopy. The shape of the Microparticles was visualized and the photographs were taken with the aid of a binocular microscope (Avanço, G.B.& Bruschi 2008).

X-ray Powder Diffraction

The X-ray powder diffraction pattern for each powder was analyzed using a D5000 XRD (Siemens, Munich, Germany). Samples were dispersed. Measurements were conducted at room temperature using Cu Kα radiation at 30 mA and 40 kV, with an angular increment of 0.04°/s and count time of 2 s. XRD samples were prepared by sandwiching the sample between the thin yellow films which is then fixed into sample holder and placed into XRD for scanning at 2θ (Mezzena M. et al 2009).

FTIR Study

The FTIR spectra of Compritol® 888 ATO, Extract: Compritol® 888 ATO (1:1) physical mixture and Extract loaded solid lipid dispersions were recorded using ATR with a Perkin Elmer Spectrum 100 FTIR spectrometer (UK) equipped with a ZnSe crystal in the 4000-650 cm-1 range. The sample (several mg) was placed on the middle on the sample stage and force applied by the top of the arm of the sample stage. After obtaining peaks with reasonable intensity, the spectra acquired were the result of averaging 4 scans at 4cm-1 resolution.(Erdal M.S.et al 2009).

Loading efficiency

One mg of Microparticles were weighed and dissolved in 0.5 ml DCM. 0.5 ml deionized water was added to this solution followed by vortexing in order to extract dry in aqueous phase. The amount of drug in samples was analyzed by HPLC method. The percentage encapsulation efficiency and drug loading can be calculated by using following formula:

% EE = (amount of drug estimated/total amount of drug added)*100

% drug loading = (amount of drug estimated/total amount of formulation)*100

RESULT AND DISCUSSION

Physical Evaluation-Calendula officinalis, Grape seed Extract & Sea buckthorn oil were visually analyzed and the result obtained are shown in Table 2.

Physical evaluation of extract showed that Calendula officinalis extract is yellow colored extract having characteristic odor. Vitis vinifer extract is having dark brown color with characteristic odor while Sea buck thorn oil is having pale yellow color with no odor.

Solubility of Extract-All extracts and oil were subjected to solubility analysis and result obtained are given in table3.

Solubility study was performed and it was observed that Calendula extract &Vitis vinifer extract was found to be soluble in Organic solvents, distill water and PBS, while Sea buck thorn oil was insoluble in water but soluble in organic solvents.

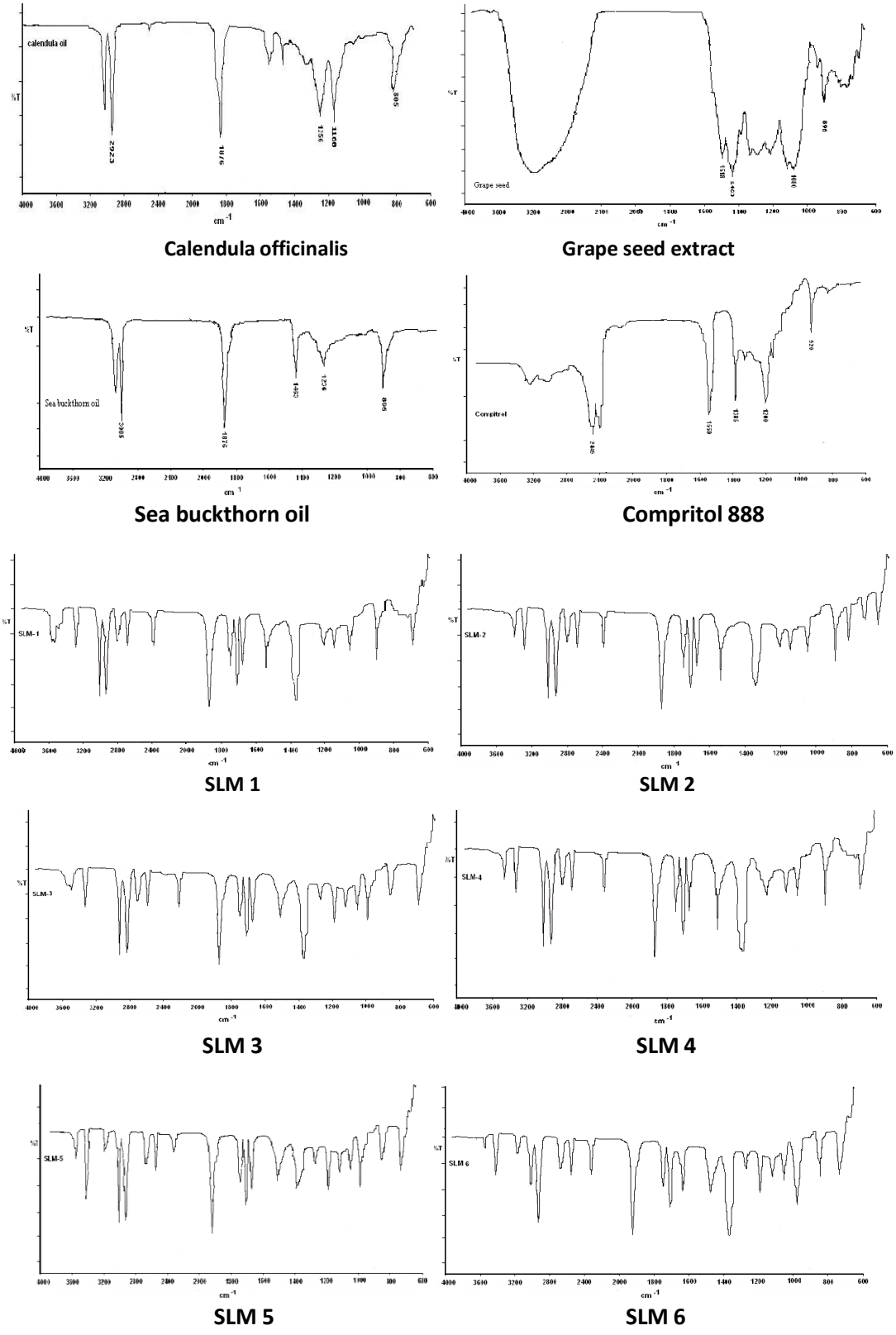

Figure 4: FTIR Images

Phytochemical studies indicate presence of constituents which are required for all activities required to treat acne.

Calendula extract showed presence of amino acids, Flavonoids, proteins, alkaloids. Flavonoids present in calendula officinalis extract possesses reported anti microbial properties and hence used in acne treatment. Vitis vinifer showed the presence of Phenolic compounds, volatile oil, Flavonoids, and amino acids. Articles reported its anti oxidant activity due to presence of Phenolic groups present in extract, while Sea buck thorn oil showed presence of Steroids, fats & oils, tannins and amino acids.

HPLC assay was performed for Quantification of active constituents present in extract required for activity using conditions given in table 4.

The unknown Extract concentration was determined by interpolating from the regression equation relating to the peak area, obtained from the set of standard solutions shown in fig 1.

Conc. found to be Calendula officinalis-0.6% Flavonoids, Grape seed -22.8% Polyphenols & sea buckthorn oil-1.66%Linoleic acid.

Particle size analysis was performed by means of optical microscopy. prepared Microparticles were filtered through Whatmann filter paper and approximately 3mg of powder is taken for analysis. Particle size analysed are given in table 5.

The choice of the emulsifier and its concentrations greatly affect the particle size of solid particles. By particle size analysis Of SLM 1,2 & 3 having different conc. of Poloxamer i.e. 0.6%,0.4% & 0.2%, Increasing the Poloxamer concentration deter-mines a significant change of the mean particle diameter. Formulation SLM3 is selected for further work because the particle size of this formulation is close to the required size. SLM 4, 5 & 6 were also subjected to Particle size analysis they also showed particle size close to particle size obtained SLM 3,and among them SLM 4 & 6 were of the size required .

Surface analysis of Solid Lipid was carried out by Scanning Electron Microscopy Images obtained after SEM are show in Figure 2.

LM1-Some Microparticles are spherical, some of them have a smooth surface, but most of them have deep folds and reefs.

SLM2-The Microparticles are more spherical than sample 1: no smooth surface, but quite rough and a lot of folding is present; a few have a donut-shape.

SLM3-Quite rough surface, not a lot of folding present, all of the Microparticles have almost spherical- shape

SLM 4-Almost all of them are spherical and have fine-smooth surface. Small folding present.

SLM 5-microparticles have spherical- shape, a fine-smooth surface, a little bit folding present but only in a few microparticles. SLM 6-A lot of folding is present, the Microparticles have early smooth surface. The majority does not have a spherical shape.

The powder x-ray diffraction pattern of Compritol 888 and different SLM prepared using Compritol 888 is shown in fig.3 Characteristic sharp peak of Compritol 888 was obtained at 4.470°, 19.458° and 21.750°. Similarly; XRD patterns of 6 different SLM preparations were also obtained. Aim to obtain XRD pattern of SLM preparations was to observe the effect and interaction of Compritol 888 with herbal extracts used. From the different patterns obtained, it can be observed that there is no significant structural change in Compritol 888 in SLM preparation as compared to Compritol 888 prior to SLM formation, only some undefined, broad, diffuse peaks of low intensities observed. Study shows Amorphous nature of Compritol Microparticles.

FTIR study of polymer and extract was performed shown in fig.4 Several intense bands 2923 cm-1, 1745 cm-1, 1464 cm-1, 1160 cm-1 in marigold have been observed. In marigold extract it was observed a strong band at 1745 cm-1 and this band corresponding for lycopene and β carotene pigments, which means that these pigments prevail in Calendula officinalis. It were observed a strong bands at 1463.89 cm-1antisymmetric band of methyl functional groups which correspond for lycopene pigments ;1464.80 cm-1, 1377.48 cm-1 germinal methyl;1238 and 1160.34 cm-1 ether linkages; 1098.44 cm-1 suggest the presence of flavones or terpenoids. The band at 1160 cm-1 can be attributed to a C–C stretching vibration of the carotenoid skeleton.

FTIR spectra of sea buckthorn some of the most significant bands are the following the band at 3485,77 cm-1is assigned to the overtone of the glyceride ester carbonyl; band appearing at 3005,61 cm-1 in the spectrum to the CH stretching of =C-H bonding; the two intensive bands at 2922,86 cm-1and 2853,64 cm-1 are assign to the aliphatic CH2 asymmetric and symmetric stretching vibration, respectively; the band at 1744,38 cm-1 is assigned to the C=O stretching vibration of the ester carbonyl functional group of the triglycerides; at 1464,76 cm-1 is observed a band which is assigned to C=H scissors deformation vibration; the band near 1377 cm-1 is assign to the bending vibration of CH2 groups; the bands at 1160,74 cm-1 and 1236,86 cm-1 are assign to the vibration of the C-O ester groups and CH2 group; band near 1117 cm-1 is associated with the stretching vibration of the C-O ester group.

FTIR of Grepe seed extract shows Glycosidic groups O-H3350 cm-1;complex C-O vibrations between 1400-1050 cm-1; phenyl rings 1515 cm-1;carbonyl substituent 1700,1626,1599 cm-1 ;strong absorption bands at 3,385,1,612,and1,067cmwere attributed to those of the characteristic functional groups of polyflavonoids;1,522and777 cm-1 were attributed to aromatic ring breathing mode and CH out-of-plane deformation with two adjacent free hydrogen atoms, respectively, indicating the prominent presence of procyanidin.

Compritol FTIR shows presence of characteristic 1739 cm-1 C=O band and 1140 cm-1 C-O band. Poloxamer FTIR shows Characteristic peaks at 2887 cm-1,1343 cm-1.

FTIR study of Microparticles indicates a complex image in which intensity of peaks were decreased due to complex formation of extracts with polymers and surfactants.

Drug Loading and Encapsulation efficiency studies were performed after optimization of Microparticles formulation i.e. SLM 3 was selected for further work on the basis of particle size and morphology. After study it was observed that SLM 3 show good Encapsulation and Loading efficiency among all were in 83.01% and 44% respectively,Results are shown in table 6.

CONCLUSION

Acne vulgaris, one of the most common diseases afflicting humanity and it is the skin disease most commonly treated by physicians. In the present scenario of drug and drug delivery development, combination of novel drug delivery loaded with herbal drug can be a very effective treatment of choice for Acne.

Sea buck thorn oil, Calendula Extract, Vitis vinifer extract were procured and characterized by phytochemical screening and physicochemical evaluation. Three preparations of Microparticles loaded with mixture of herbal extracts & oil were formulated. These Microparticles then characterized by various methods like Particle size,SEM, XRD etc. and on the basis of this characterization Microparticles with lowest conc. of emulsifier was selected for further work. Using the formula with lowest conc. of emulsifier three Microparticles were prepared with varied conc. of extracts and oil and then Evaluated on the basis of Loading and encapsulation efficiency, particle size, XRD, SEM etc.

Although various topical herbal formulations for acne are available in the market, this work makes use of Calendula extract, Vitis vinifer extract and sea buckthorn oil for the first time in the developed formulation. It is clear from above evidences that Calendula officinalis extract, Vitis vinifer extract and sea buck thorn oil loaded Solid lipid Microparticles are highly promising Carriers for acne treatment.

ACKNOWLEDGEMENT

I feel honored to acknowledge my immense gratitude to my precious institution Swami Vivekanand College of Pharmacy, Indore (M.P.), for providing me the means of attaining my most cherished goal. I am thankful to Amruta Herbal Pvt Ltd, Indore for providing Herbal extracts and DAVV, Indore for X-ray and SEM study.

REFERENCES

Adityan B et al;"Scoring systems in acne vulgaris";Indian J Dermatol Venereol Leprol ; 2009;75 (3); 323–6.

Ashkenazi, H.; Malik, Z.; Harth, Y.; Nitzan, Y.Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. FEMS Immunol. Med. Microbiol. 2003, 35 (1), 17–24.

Avanço, G.B.& Bruschi, M.L.; Preparation and characterisation of ethylcellulose microparticles containing propolis; Journal of Basic and Applied Pharmaceutical Sciences,2008; v. 29, n.2, p. 129-134, 2008.

Babu S.S.; Book for "Herbal Cosmetics";Pustak mahal prakashan,Delhi;6-8.

Christie W.W.; analysis of fatty acids by high performance Liquid chromatography; lipidlibrary.aocs.org,25 july 2011;1-4.

Dieneka V.I. et al;Reversed phase HPLC in the Analysis of plant oils Monitoring of Authenticity and detection of Adulteration of Sea buckthorn oil;Pharmaceutical Chemistry journal,2009;vol.43;1;31-33.

Erdal M.S.et al.; " Preparation and in vitro evaluation of indomethacin loaded solid lipid Microparticles"; Acta Pharmaceutica Sciencia,2009;5;: 203- 210.

Fonseca Y.M.et al;Determination of rutin and narcissin in marigold extract and topical formulations by liquid chromatography: applicability in skin penetration studies; Quím. Nova,2010; vol.33 no.6

Fuleki T,and Silva RD: Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. J Agric Food Chem,1997;45:1156–1160.

Higaki, S. Lipase inhibitors for the treatment of acne.J. Mol. Catal., B Enzym. 2003, 22 (5–6), 377–384.

Khandelwal k. R.,"Practical P'Cognocy"; Nirali prakashan, Pune;2008;19thedition;142-48.

López M.et al; HPLC-analysis of polyphenolic compounds in spanish red wines and determination of their antioxidant activity by radical scavenging assay;Food Innova,2010;2-4.

Madhav N.S. & Kala S.;"Review on Microparticulate drug delivery system"; International Journal of PharmTech Research;,July-Sept 2011;Vol.3(3) 1242-1254.

Mezzena M. et al; "Solid Lipid Budesonide Microparticles for Controlled Release Inhalation Therapy" ;The AAPS Journal,;December 2009 ;Vol. 11(4) ;771-772

Rodriguez D.et al; Principal component analysis of the polyphenol content in young red wines; Food Chemistry, 2002;78; 523-532.

Sanna V. et al; "Preparation and In Vivo Toxicity Study of Solid Lipid Microparticles as Carrier for Pulmonary Administration";AAPS PharmSciTech;2004; 5(2); 27.

Webster GF.; "The pathophysiology of acne":Cutis; 2005 Aug ;76(2):4-7.