



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 232-240
Received: 02-05-2015
Accepted: 04-06-2015

Alisha Pereira
SVKM's Dr. Bhanuben Nanavati
College of Pharmacy, SVKM
College Campus, V.M. Road, Vile
Parle (W), Mumbai 400056,
India.

Rashmi Mallya
SVKM's Dr. Bhanuben Nanavati
College of Pharmacy, SVKM
College Campus, V.M. Road, Vile
Parle (W), Mumbai 400056,
India.

Formulation and evaluation of a photoprotectant cream containing *Phyllanthus emblica* extract-phospholipid complex

Alisha Pereira, Rashmi Mallya

Abstract

Photoprotectives in skin-care is essential to prevent oxidative stress induced by long term exposure to UV. *Phyllanthus emblica* fruit extract contains polyphenols that are potent antioxidants. But polyphenols have limited penetration into skin therefore its topical use, as a photoprotectant, is ineffective. This study aims to develop a phospholipid complex of amla extract that enhances the delivery of polyphenols into the skin. The amla extract phospholipid developed, had an entrapment efficiency of 94.03 ± 0.10 %. The characterization studies showed that constituents of amla extract successfully formed a complex with phospholipids. The complex was incorporated into a cream formulation, which was found to be stable. The *ex-vivo* diffusion studies of cream showed that administration of the amla extract via phospholipid complexes gave better skin retention compared to the conventional cream. Thus, phospholipid complex of amla extract gave a prolonged antioxidant effect compared to the conventional cream.

Keywords: Amla, phospholipid-complex, cosmetic cream, antioxidant, photoprotectant, SPF.

1. Introduction

Long term exposure of human skin to ultraviolet rays produces reactive free radicals and induces oxidative stress on skin leading to the development of erythema, sunburn, hyperplasia, immunosuppression, photoaging, melanogenesis and DNA damage that can lead to skin cancer [1].

The use of chemical sunscreens as photoprotectives is less desirable now-a-days because it can cause photo contact dermatitis, skin tumors and estrogen like effects on prolonged use. Hence the use of photoprotectives from natural origin is gaining attention to prevent the deleterious effects of UV rays [2]. The polyphenolic compounds such as flavonoids, phenolic acids, tannins and their hydrolysable and condensed polymers due to their antioxidant property can be used as photoprotectives [3].

The fruits of *Phyllanthus emblica* (Phyllanthaceae) commonly known as amla, is rich in polyphenols including tannins, phenolic glycosides and flavonoids. It also contains terpenes, sterols, and essential trace elements, vitamins (mainly vitamin C) and amino acids [4]. Amla extract is known to exhibit potent antioxidant properties and protect skin fibroblasts against oxidative stress. It also induces fibroblast proliferation, controls collagen metabolism and promotes production of procollagen [5]. Gallic acid, the active constituent of amla has a strong antioxidant activity, acts as a depigmenting agent and inhibits UV induced immunosuppression [6].

The delivery of polyphenols topically is not effective because it has limited penetration into skin. Thus it requires the use of penetrations enhancers or formulation base that enhance its penetration [7]. A phyto-phospholipid complex (Phytosome) is a system where plant extracts are incorporated into phospholipids (phosphatidylcholine or phosphatidylserine). The presence of phospholipids allows higher adhesion of the complex to the skin surface and improved topical absorption due to its surfactant and lipophilic nature. It also improves hydration and collagen structure. Thus use of phospholipid complex of bioactive compounds topically provides improved local action and enhances activity by improving absorption and delivery to tissues [8].

The present study thus aims to develop a cream containing amla extract phospholipid complex to be used as antioxidant cosmetic formulation for photoprotection.

2. Materials and methods

Amla powder was purchased from the local market in Mumbai and authenticated by Dr. Bindu Gopalkrishnan, Botany department, Mithibai College, Vile Parle (W), Mumbai. A specimen of

Correspondence

Alisha Pereira
Address for correspondence:
SVKM's Dr. Bhanuben Nanavati
College of Pharmacy, SVKM
College Campus, V.M. Road, Vile
Parle (W), Mumbai 400056,
India.

same has been deposited at Department of Botany, Mithibai College. (Specimen no- MIT 0025), Soya phosphatidylcholine (Leciva S-70) was obtained as a gift sample from VAV Life Sciences, Mumbai. The major equipment used in the study were UV-Visible spectrophotometer (Shimadzu UV-1800 version 2.33), FT-IR Spectrometer (Bruker IFS-55, Switzerland), Scanning Electron Microscope (Zeiss Ultra-55) and Modified Franz diffusion apparatus (DBK- diffusion cell apparatus, India)

2.1. Preparation and Evaluation of methanolic extract of Amla

Methanolic extract of amla powder was obtained after soxhlet extraction. Resulting extract was concentrated using distillation apparatus and evaporated in a porcelain dish on electric water bath at 40 °C to remove solvent. Percent yield of the extract was calculated and preliminary phytochemical screening carried out.

The concentration of phenolics and flavonoids in plant extract was determined spectrophotometrically [9]. The content of total phenols was determined using Folin-Ciocalteu's reagent at 765 nm. The result was expressed as gallic acid equivalent (mg) per 100 g extract. (Mg of GA/g of extract). Total flavonoid content was measured by the aluminium chloride colorimetric assay. The absorbance was measured against the blank at 510 nm using rutin as standard. The total flavonoid content was expressed as rutin equivalent (mg of rutin/g of extract).

As a measure of free radical scavenging property, antioxidant activities of extracts were determined using hydrogen peroxide scavenging assay [10] and 2, 2'-diphenyl-1-picryl hydrazyl (DPPH) assay [11]. Ascorbic acid was used as standard for comparison. Results were expressed in terms of IC₅₀ and percent H₂O₂ scavenging and DPPH inhibitory activity.

2.2. Preparation of amla extract-phospholipid complexes

Solvent evaporation technique [12] was used for the preparation of complexes. Phospholipid and amla extract at various molar ratios 1:1, 1:2, 1:3, and 2:1 were placed in a 250ml round-bottom flask with dichloromethane or methanol as solvent and refluxed at 50 °C for 3 hours. The resultant clear mixture was evaporated on water bath and 20 ml of n-hexane was added to it with stirring. The precipitate was filtered and dried under vacuum and placed in desiccator overnight and percent yield was calculated.

2.3. Optimization of complexes

Entrapment efficiency and drug content of the complexes were studied to optimize the ratio of drug to phospholipid in formulation.

For entrapment efficiency studies, 10mg of complex in 10 ml phosphate buffer was ultra-centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant was decanted and the sediment was dispersed in 10ml methanol and sonicated for 20 mins. The absorbance was taken at 273nm and entrapment efficiency was calculated using the formula

$$\% \text{ Drug entrapped} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

Drug (gallic acid) content in the phospholipid complex was determined spectrophotometrically at 273 nm using methanol as solvent and phosphatidylcholine solution in methanol as blank.

2.4. Evaluation of the optimized extract-phospholipid complex

Suspension of the complex in distilled water was applied on a silicon wafer and was dried in oven. It was transferred to the microscope (Zeiss Ultra-55) and Scanning Emission Microscopy images were captured and observed.

FTIR spectra and Differential Scanning Calorimeter (DSC) studies of amla extract, phosphatidylcholine, amla extract: phosphatidylcholine complex (1:1), and physical mixture of amla extract: phosphatidylcholine (1:1), were conducted. Pellets for FTIR studies were made using dry crystalline KBr and sample in a ratio of 1:100 and pressing in hydraulic press. DSC samples were sealed in an aluminium crimp cell and heated at a speed of 10 °C/minute from 30 to 300 °C in nitrogen atmosphere (60 mL/minute).

2.5. Formulation of cream bases

Ingredients listed in Table 1 were accurately weighed and placed in separate beakers (for oil phase and water phase). The beakers were heated to 70 °C on a water bath. On complete melting of the ingredients, the contents of the water phase were added to the oil phase with constant stirring. The resulting emulsion was stirred until it cooled and congealed. 1% amla-phosphatidylcholine complex in (1:1 ratio) was incorporated into the base by shear.

Conventional (1%) amla extract cream was prepared by incorporating desired quantity of the extract in the water phase and then adding it to the oil phase with stirring using the same method as above.

Table 1: Ingredients for preparation of cream formulations

Ingredients	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
Stearic acid	24	13	4	20	20	10	20
Mineral oil	-	-	-	-	-	3.5	-
IPM	-	-	-	20	2	-	-
TEA	-	-	-	1.2	1.2	-	1.2
Petroleum jelly	-	-	1	-	-	-	2
Cetyl alcohol	-	1	5	-	-	2	-
KOH	1	0.9	1	-	-	-	-
Glycerin	11	10	5	-	-	-	-

GMS	-	1	-	-	-	-	-
Sodium metabisulphite	-	-	-	-	-	0.1	-
EDTA	-	-	-	-	-	0.1	-
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Water	q.s. to 100gms	q.s. to 100gms	q.s. to 100gms	q.s. to 100gms	q.s. to 100gms	q.s. to 100gms	q.s. to 100gms
Active	1	1	1	1	2	1	1

2.6. Evaluation and Stability studies of cream

The cream formulations were evaluated at zero time and over a period of 3 months at specific time intervals for organoleptic properties (colour, homogeneity and phase separation) and physico-chemical evaluation (pH, spreadability, viscosity, drug content and antioxidant activity). Stability studies of 1% amla extract-phospholipid cream were carried out as per ICH guidelines after packing it suitable glass containers and placing in stability chambers.

2.7. Ex vivo skin diffusion studies

The study was performed using modified Franz diffusion cell using pig ear skin as membrane (COLIPA, 1997) [13]. Comparative studies were carried out between amla extract-phospholipid complex cream (1%) and conventional amla extract cream (1%). The skin on both sides of the ear was cut open and was cleaned of underlying tissues, fat and hair. The membrane was then stored in the refrigerator until use. Before the study, membrane was allowed to attain room temperature in phosphate buffer (pH 7.4) for an hour.

The receptor compartment with volume of approximately 22 ml was filled with phosphate buffer. Pig skin membrane (dermal side down) was clamped between the donor and receptor compartments which had a diffusion area of 1.77cm². 1gm of each formulation was weighed and applied on the membrane and the donor chamber was covered to prevent evaporation. The receptor solution was stirred continuously at 210 rpm with the help of a magnetic stirrer at a temperature of 37 °C maintained by circulating water externally. 2 ml aliquots were withdrawn through the sampling port at 1, 2, 3, 6, 8, 12, and 24 hours and the cells were replaced with same quantity of fresh buffer each time to maintain a constant volume. Gallic acid content in each aliquot was then measured spectrophotometrically at 273 nm and calculated using gallic acid standard curve taken in phosphate buffer.

The gallic acid retained in the skin was determined at the end of 24 hours after *ex-vivo* study. The skin was detached from the diffusion cell, excess formulation was washed off and the pig skin was cut into pieces and soaked in methanol (10 ml) [14]. It was then homogenized at 24,000 rpm for 5 minutes. The solution filtered and centrifuged at 15,000 rpm for 30 minutes at 4 °C. The amount of gallic acid retained was measured spectrophotometrically at 273 nm and calculated from the gallic acid standard curve.

2.8. SPF evaluation

The SPF study of the 1% amla-phospholipid complex cream was carried out at Kelkar Education Trust's Scientific Research Centre, Mulund. (Report no: SRC/CD/1568)

The study was performed by the transmittance measurement of the cream. The UV-2000S Ultraviolet Transmittance Analyzer

measured the sun protection factor of the cream over a wavelength range of 290 nm- 400 nm. Approximately 110 mg of the prepared investigational sample was applied and spread on a 56cm² area of a transpore tape to obtain an even sample film thickness of 2µl/cm². The sample was then exposed to xenon flash lamp for determining the sun protection factor and boots star rating.

The SPF value of the formulation was then calculated by the following formula:

$$SPF = \frac{\int_{290}^{400} E_{\lambda} S_{\lambda} d\lambda}{\int_{290}^{400} E_{\lambda} S_{\lambda} T_{\lambda} d\lambda}$$

Where, E (λ) is the erythema action spectrum.

S (λ) is the solar spectral irradiance.

T (λ) is the spectral transmittance of the sample.

3. Results and discussion

The yield of methanolic extract of amla was 43.87%. Phytochemical analysis of the extract showed the presence of carbohydrates, flavonoids, tannins and phenols. The total phenolic content in extract calculated (n=3) from regression equation of the standard curve of gallic acid ($y = 0.0153x - 0.0131$, $r^2=0.996$) was found to be 179.94±0.58 mg GAE/g of extract. The total flavonoid content in the methanolic extract was found to be 129.53±0.88 mg of rutin/g of extract as calculated (n=3) from regression equation of the standard curve of rutin ($y = 0.0015x + 0.0057$, $r^2=0.999$).

The extract was capable of scavenging hydrogen peroxide and inhibiting DPPH in a concentration-dependent manner as seen in fig.1 and fig.2. The IC₅₀ of extract in H₂O₂ scavenging assay was 39.5±0.07 µg/ml in comparison to standard ascorbic acid 32±0.18 µg/ml and IC₅₀ of the extract for DPPH assay was 47±0.09µg/ml when compared to ascorbic acid 35±0.21µg/ml. The observed antioxidant effect can be attributed majorly to the phenolic compounds. These act as free radical scavengers by virtue of their hydrogen-donating capacity and ability to transfer of hydrogen atom or electron [16].

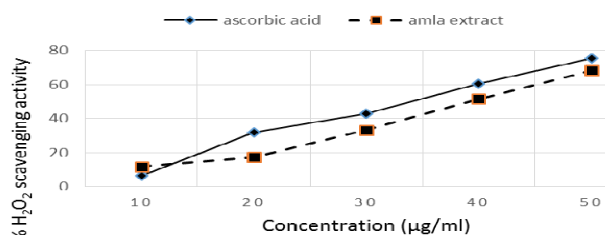


Fig 1: Hydrogen peroxide free radical scavenging activity of amla extract

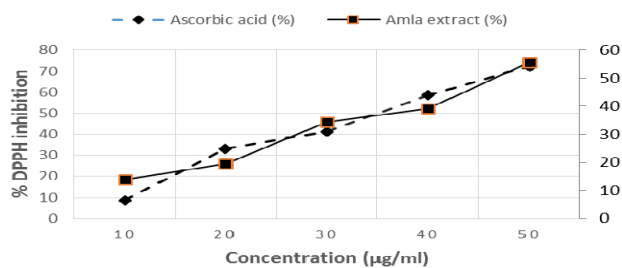


Fig 2: DPPH radical scavenging activity of amla extract Optimization of amla extract-phospholipid complexes

Yield of complexes via solvent evaporation by dichloromethane and methanol was found to be 12% and 78% respectively. Hence, methanol as solvent was preferred for the preparation of complexes different ratios of extract and phospholipids.

The results of entrapment efficiency and drug content of complexes is tabulated in Table 2. The complex with 1:1 ratio of extract and phospholipid was used for further studies because it showed a higher entrapment efficiency of 94.03 ± 0.10 % and drug content of 97.60 ± 0.09 % compared to the other ratios prepared.

Table 2: Entrapment efficiency and drug content of complexes

No.	Complex ratio	Entrapment efficiency (%)	Drug content (%)
1	1:1	94.03 ± 0.10	97.60 ± 0.09
2	1:2	87.0 ± 0.040	85.30 ± 0.01
3	1:3	79.43 ± 0.104	92.64 ± 0.13
4	2:1	90.79 ± 0.8	63.49 ± 0.18

3.1. Evaluation of the optimized extract-phospholipid complex (1:1)

The SEM imaging of 1:1 complex revealed well formed, discrete vesicles (fig.3). When swirled in distilled water, complexes formed ordered phytovesicular structures with no signs of aggregation or decomposition morphologically.

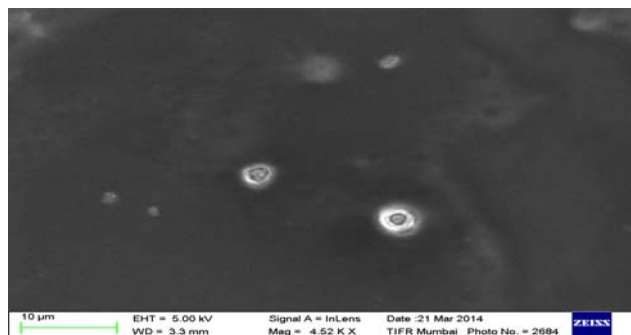


Fig 3: SEM generated picture of the 1:1 ratio complex (at 4.52K X)

IR spectra of amla extract, phosphatidylcholine, physical mixture (1:1), and the complex (1:1) were as shown in fig.4a-fig.4d. Shifting of the $-OH$ group (3400 cm^{-1}) of amla extract to a lower wave number in the complex spectra indicated a formation of hydrogen bond between OH of extract and phospholipid phosphate. The $P-O-C$ bond of phosphatidylcholine shifted from 1073 cm^{-1} to a lower wavenumber of 1032 cm^{-1} , whereas, the absorption peak of choline at 956 cm^{-1} shifted to a higher frequency with decreased intensity in the complex, showing interaction at the level of choline moiety. The $P=O$ bond at 1224 cm^{-1} disappeared in the complex spectra indicating that some groups of the amla extract interacted with the $P=O$ group of phospholipid. There was also a slight difference between the physical mixture and the complex in the wavelength range from 1200 cm^{-1} to 960 cm^{-1} corresponding to the region of the phosphate group. The spectra of the physical mixture showed an additive effect, in which the characteristic absorption bands of phospholipid and amla extract were seen at 1073 cm^{-1} and 2352 cm^{-1} . These observations were in accordance with those of Hou *et al.* [17] which suggest that hydrogen bonding and other weak physical interactions between the extract and phospholipid play an important role during the formation of the complex.

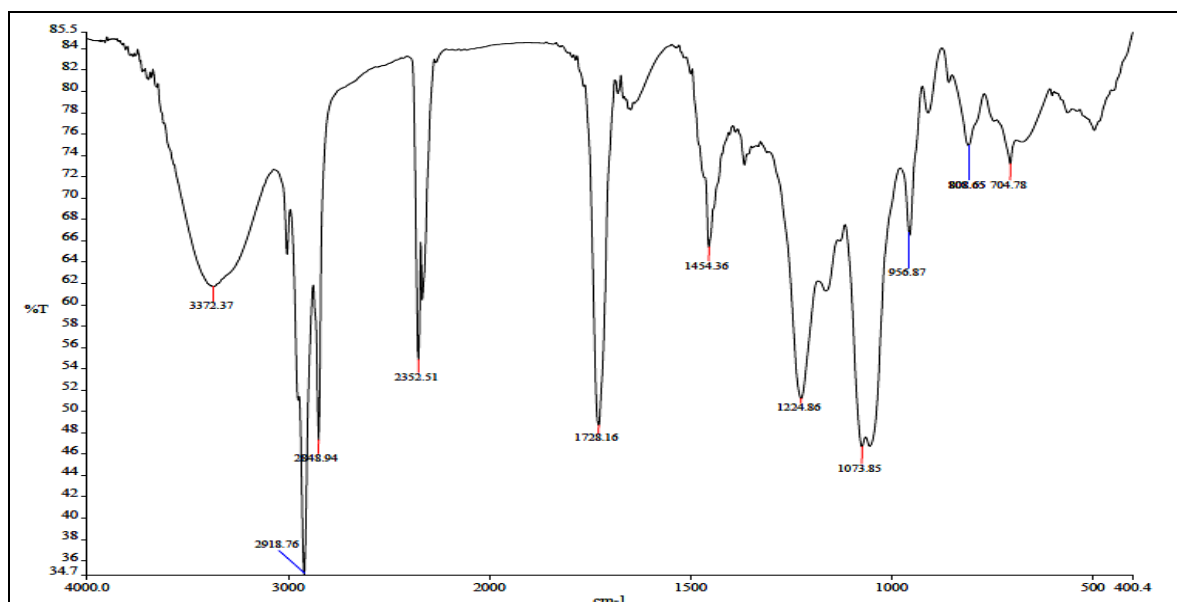


Fig 4a: FT-IR spectra of phosphatidylcholine

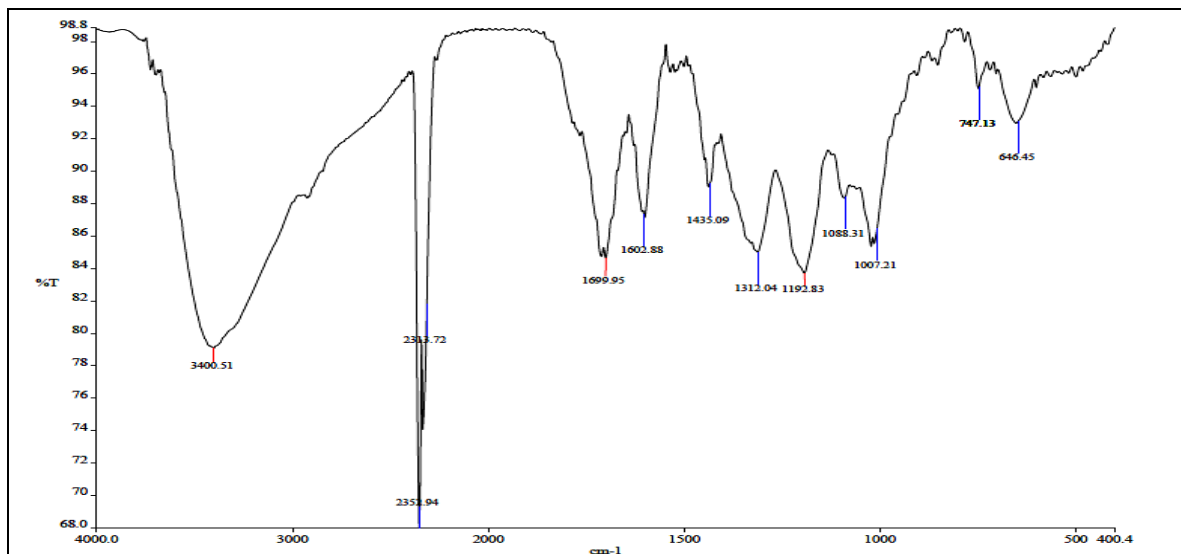


Fig 4b: FT-IR spectra amla extract

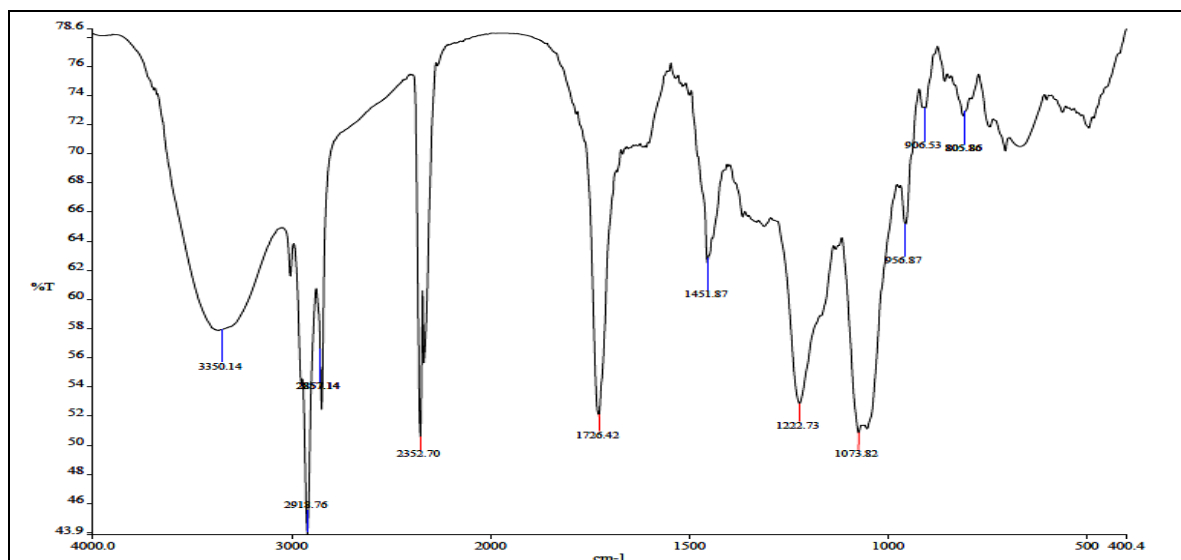


Fig 4c: FT-IR spectra physical mixture (1:1)

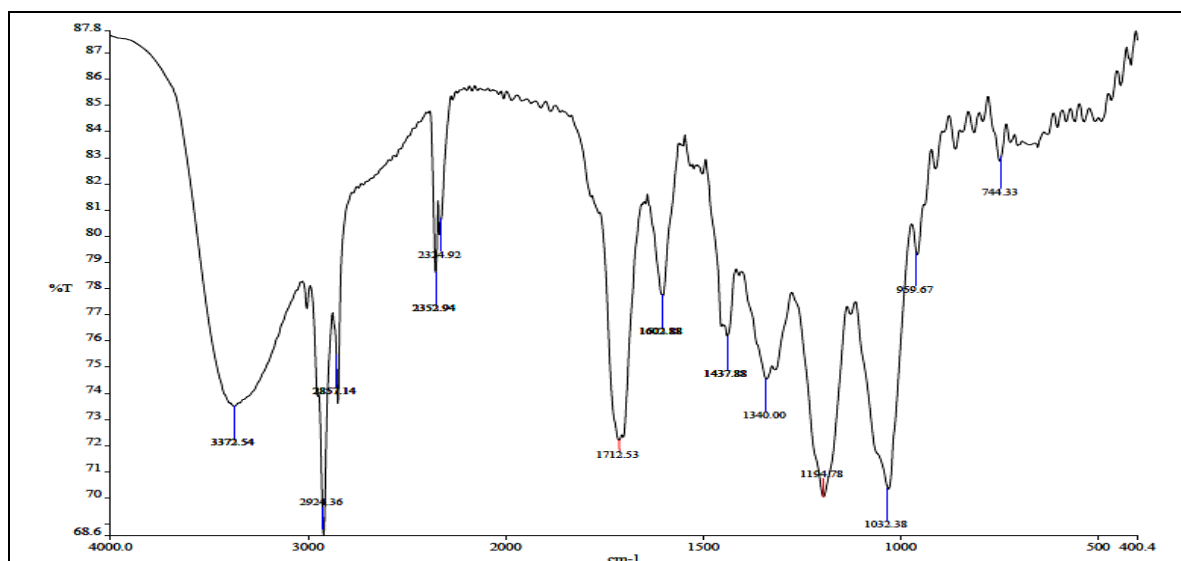


Fig 4d: FT-IR spectra of amla extract: phosphatidylcholine complex (1:1)

Phosphatidylcholine complex (1:1)

Thermal analysis is one of the crucial tools to characterize the solid state of matter, particularly in complex form. From fig.5a-fig.5d it is observed that phospholipid thermogram demonstrated a sharp, pointed endothermic peak at 170.0 °C that could be attributed to the transition from gel state to liquid crystal state. The amla extract thermogram shows a sharp endothermic peak at 151.8 °C, corresponding to extract melting. In the physical mixture thermogram, the endothermic

peaks of both the extract and the phospholipid are still detectable but shifted towards lower temperatures (132.8 °C and 141.0 °C, respectively). On the other hand, the DSC thermogram of the amla extract-phospholipid complex revealed the disappearance of the extract endothermic peak, with a broad endothermic peak appearing at 139.8 °C. This peak may be attributed to the formation of a new complex peak. These findings can explain complex formation by interactions including H-bonds or Van der Waals forces [18].

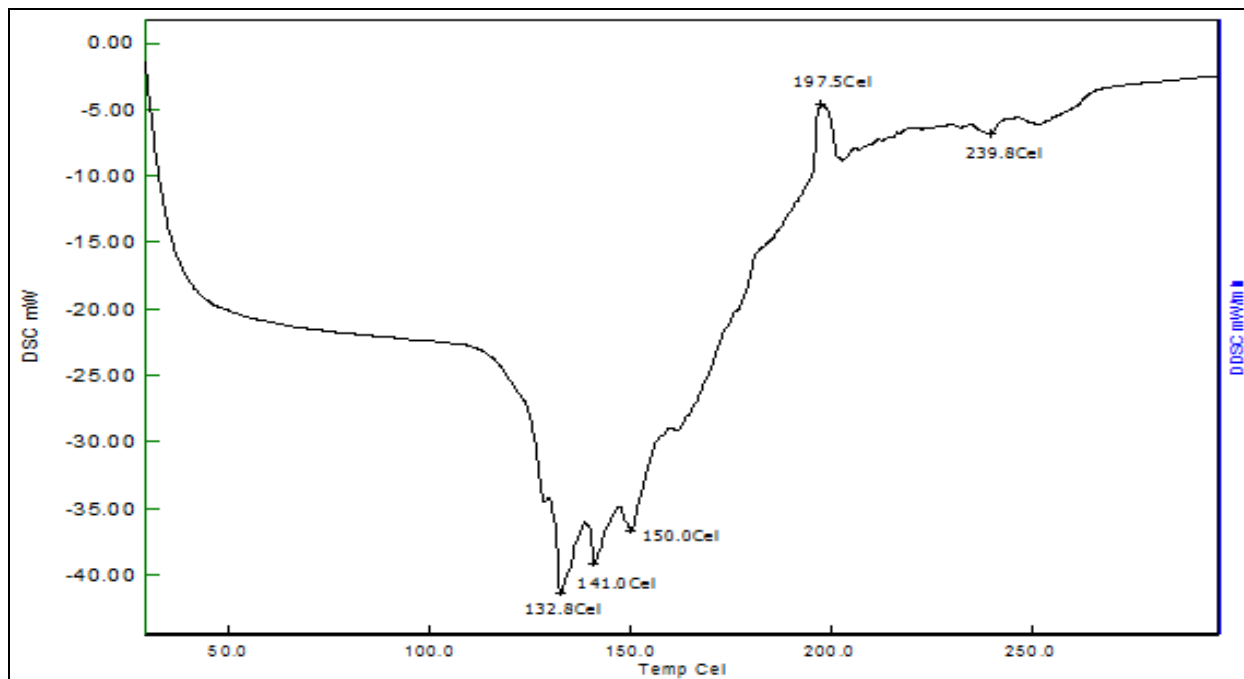


Fig 5a: DSC thermogram of physical mixture (1:1)

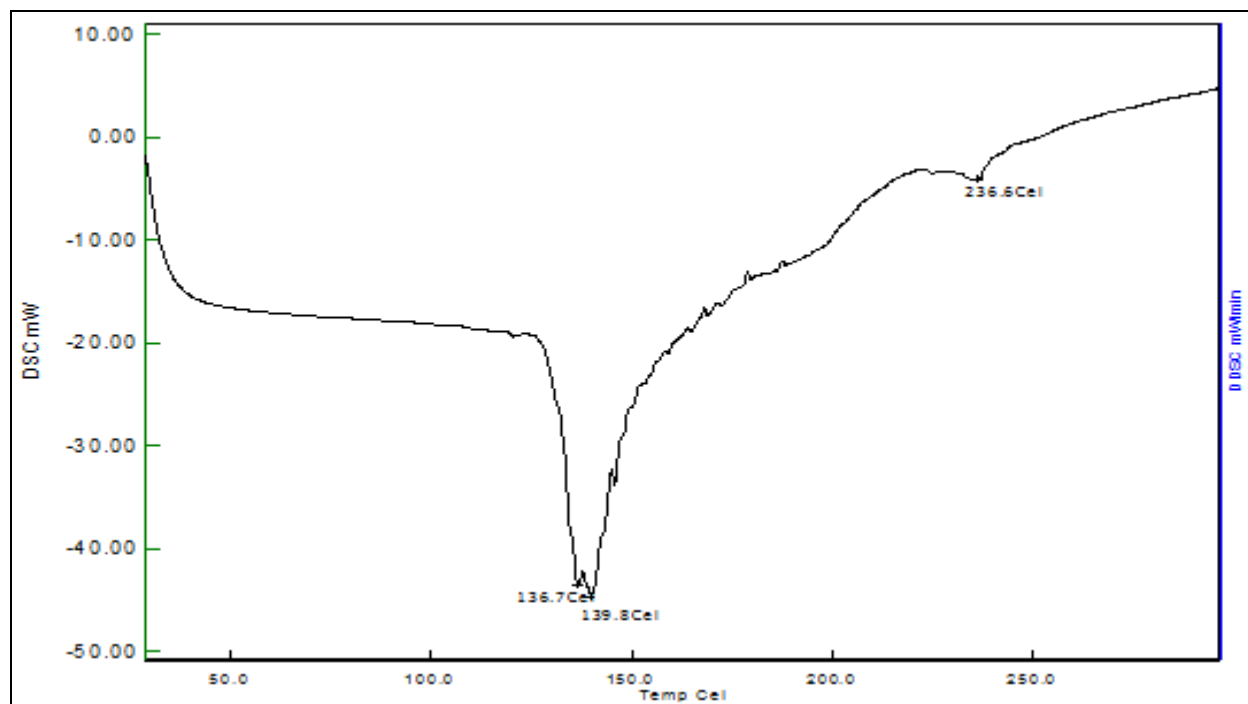


Fig 5b: DSC thermogram of amla extract: phosphatidylcholine complex (1:1)

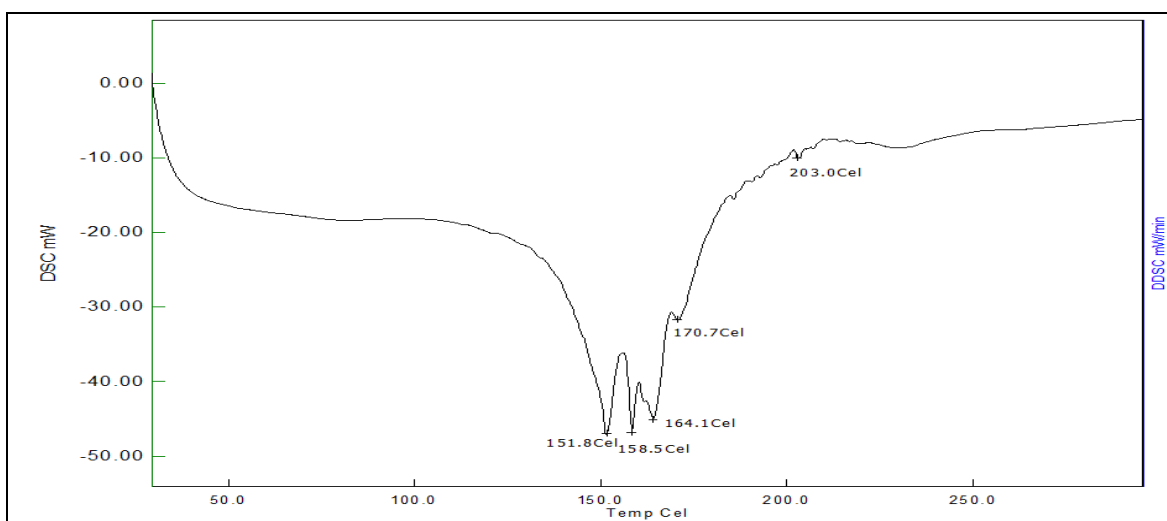


Fig 5c: DSC thermogram of amla extract

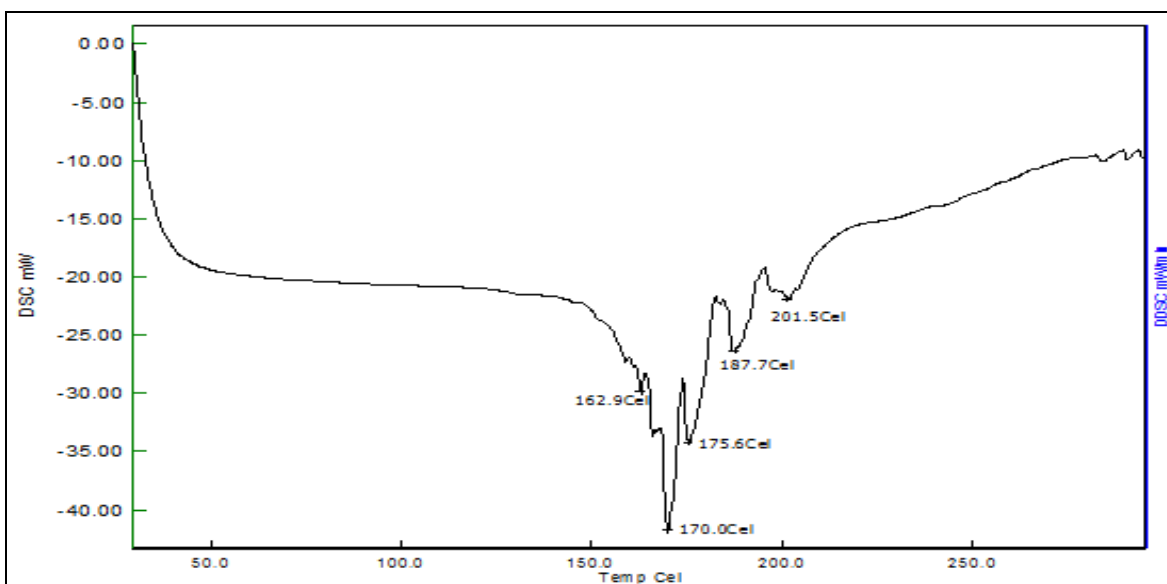


Fig 5d: DSC thermogram of phosphatidylcholine

3.2. Evaluation of the optimized cream

Batches 1-3 and 6 were unstable and caused foaming and color change on standing for a week of preparation. Batches 4 and 5 lost moisture and dried up within 3 days of preparation. Batch 7 gave a product which was stable for over a week after preparing it. Hence, the formula was chosen for final preparation of the amla extract-phospholipid complex cream. Thus, the bases with triethanolamine stearate emulsifier (formed *in-situ*) were more compatible with the complex than those with potassium stearate emulsifier (formed *in-situ*). Also, compositions with petroleum jelly showed a better emollient property than those using isopropyl myristate (IPM), mineral oil and glycerine.

3.3. Ex vivo diffusion studies

Percent of gallic acid released at each time point was calculated by a gallic acid standard with a curve as shown in fig. 6 with a regression equation of $y = 0.0888x - 0.0166$ and $R^2 = 0.9998$

The cumulative percentage of gallic acid in the receptor fluid over 24 hours was higher with the conventional extract cream ($68.33 \pm 0.39\%$) than with extract-phospholipid complex cream ($41.67 \pm 0.62\%$) and 1% amla extract-phospholipid complex cream showed higher retention of gallic acid in the skin ($22.8 \pm 0.54\%$) compared to the conventional extract cream ($4.9 \pm 0.06\%$) (Fig. 7). Thus complex cream showed higher localization of gallic acid in the skin as compared to conventional extract cream. Such accumulation of the extract/phytoconstituent in the skin acting as depot for a longer time is good because it helps sustain release of actives in the epidermis over a period of time and makes it suitable for cosmetic products that are meant to act upon the skin. This property of the extract-phospholipid cream can be attributed to the fact that since phospholipids are main components of stratum corneum, they can transport drugs to target skin cells [19].

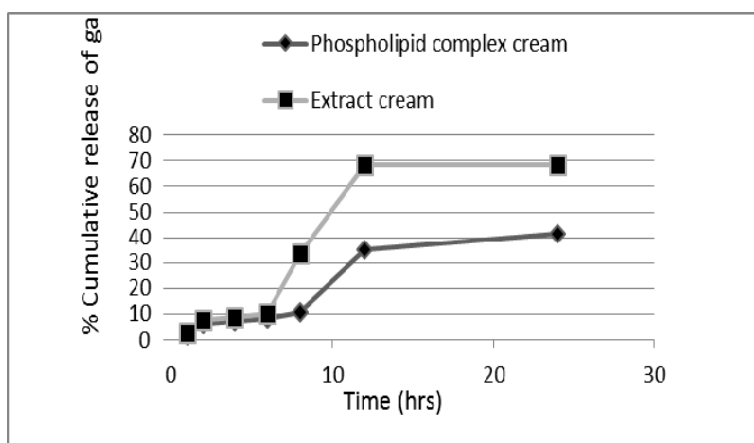


Fig 6: Ex vivo diffusion studies

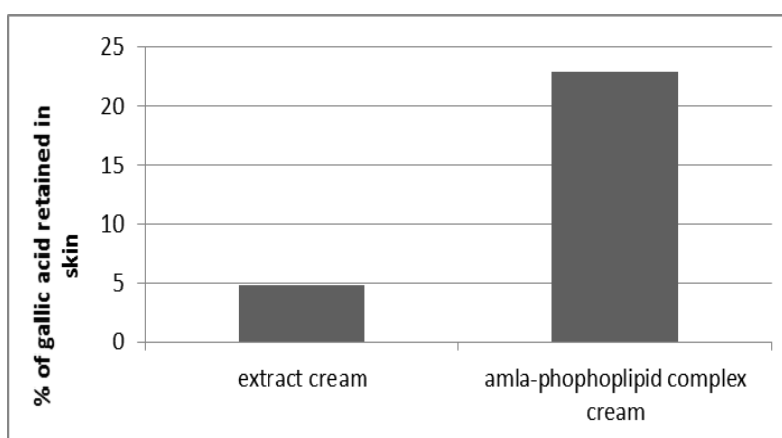


Fig 7: Analysis of Gallic acid retained in skin

3.4. Stability evaluation

The result of stability studies is shown in Table 3. The visual appearance of cream formulation was checked at the time of preparation and at the end of every month until 3 months

period. There was no significant difference in visual appearance, viscosity, and drug content and *in-vitro* antioxidant activity at the end of three months period. Thus the formulation was found to be stable under specified conditions.

Table 3: Stability study results

Time	0 month	1 st month		2 nd month		3 rd month	
Temperature	-	RT	40 °C/ 75%RH	RT	40 °C/ 75%RH	RT	40 °C/ 75%RH
Colour and Homogeneity	Light brown and Intact throughout the entire study period						
pH	7.69 ±0.65	7.60 ±0.54	7.53 ±0.52	7.60 ±0.85	7.49 ±0.78	7.56 ±0.54	7.34 ±0.60
Viscosity (cp at 2.5 rpm)	1095 ±0.45	1087 ±0.42	1079 ±0.54	1084 ±0.87	1073 ±0.92	1079 ±0.58	1068 ±0.54
Spreadability (gm.cm/sec)	13.2 ±0.12	13.6 ±0.24	13.9 ±0.52	13.8 ±0.46	14.2 ±0.53	14.4 ±0.64	14.7 ±0.82
Drug Content (%)	97.86 ±0.04	97.75 ±0.09	97.72 ±0.010	97.73 ±0.008	97.70 ±0.19	97.64 ±0.49	97.62 ±0.15
Antioxidant activity (%)	96.07 ±0.09	95.78 ±0.07	95.74 ±0.086	95.04 ±0.010	94.86 ±0.007	94.92 ±0.08	94.72 ±0.14

3.5. SPF evaluation

Amla extract-phospholipid complex cream gave an average SPF value of 2.09 with no boot star rating (Table 4). The principle of the SPF study was based on the sample transmittance measurement, where transmittance is defined as the ratio of the illumination passed through a sample to the

illumination impinging on the sample. Thus the addition of amla extract phospholipid complex to a broad spectrum sunscreen formulation may help decrease damage caused by UV compared to sunscreen alone by its antioxidant effect [20].

In conclusion, the present study showed that the formulation containing phospholipid complex of amla, a potent natural

antioxidant provides an active skin delivery system compared to the conventional formulation. Also, phospholipid in the complex helps to nourish skin by improving its hydration, and maintain integrity of the collagen structure, further contributing to its healthy appearance. This enhanced skin delivery would help the herbal extract in acting against effects of UV radiation at an early stage and thus preventing any further damage to the skin. Thus, the phospholipid complex of amla extract can either be used alone or as an additive to a sunscreen formulation, consequently providing a good rationale for the development of the complex into a novel photoprotective cosmetic formulation with promising effects.

Table 4: SPF evaluation results of cream formulation containing amla extract phospholipid complex

Parameters				Average values
Scans	1	2	3	
SPF	2.14	2.08	2.07	2.09
Standard deviation	0.06	0.03	0.02	0.03
UVA/UVB Ratio	0.563	0.550	0.546	0.553
Critical Wavelength	382.33	382.17	382.00	382.16
Boots Star Rating	No rating	No rating	No rating	No rating

4. Acknowledgements

We thank VAV Lifesciences, Mumbai for their generous gift sample of phosphatidylcholine, Kelkar Education Trust's Scientific Research Centre, Mulund, for their help in completing the SPF studies and SVKM's Dr. Bhanuben Nanavati College of Pharmacy for the facilities provided to conduct the research work.

5. References

- Afag F, Mukhtar H. Effects of solar radiation on cutaneous detoxification pathways. *J Photochem. Photobiol.* 2001; 63:61-9.
- Kumar S, Gupta RN. Safety and regulatory issues on sunscreen products in India. *Arch Appl Sci Res* 2013; 5(2):145-153.
- Saraf S, Kaur CD. Phytoconstituents as photoprotective novel cosmetic formulations. *Phcog Rev* 2010; 4:1-11.
- Zhu X, Wang J, Ou Y, Han W, Li H. Polyphenol extract of *Phyllanthus emblica* (PEEP) induces inhibition of cell proliferation and triggers apoptosis in cervical cancer cells. *Eur. J Med. Res.* 2013; 18:46.
- Fujii T, Wakaizumi M, Ikami T, Saito M. Amla (*Emblica officinalis* Gaertn.) extract promotes procollagen production and inhibits matrix metalloproteinase-1 in human skin fibroblasts. *J Ethnopharmacol.* 2008; 119(1):53-7.
- Kumar KJ, Vani MG, Wang SY, Liao JW, Hsu LS, Yang HL *et al.* *In vitro* and *in vivo* studies disclosed the depigmenting effects of gallic acid: a novel skin lightening agent for hyperpigmentary skin diseases. *Biofactors* 2013; 39(3):259-70.
- Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: Anti-inflammatory, anti-oxidant and DNA repair mechanisms. *Arch. Dermatol Res* 2010; 302(2):71-83.
- Bombardelli E, Curri SB, Della LR. Complexes between phospholipids and vegetal derivatives of biological interest. *Fitoterapia* 1989; 90(1):1-9.
- Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J Chem. Technol. Metall.* 2005; 40(3):255-260.
- Shirwaikar A, Prabhu KS, Punitha ISR. *In-vitro* antioxidant studies of *Sphaeranthus indicus* (Linn). *Ind. J Exp. Bio.* 2006; 44:993-996.
- Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989; 10:1003-1008.
- Freag MS, Elnaggar Y, Abdallah OY. Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and *ex vivo* permeation. *Int. J Nanomedicine.* 2013; 8:2385-2397.
- COLIPA. European Cosmetic, Toiletry and Perfumery Association. Guidelines for percutaneous absorption/penetration, 1997.
- Touitou E, Meidan VM, Horowitz E. Methods for quantitative determination of drug localized in the skin. *J Control Release.* 1998; 56:7-21.
- Ravikumar P, Mallya R. Development and evaluation of a polyherbal topical formulation, JPR: BioMedRx: An International Journal. 2013; 1(7):637-640.
- Saha S, Verma RJ. Antioxidant activity of polyphenolic extract of *Terminalia chebula* Retzius fruits, *Journal of Taibah University for Science*, 2014. <http://dx.doi.org/10.1016/j.jtusc.2014.09.003>.
- Hou Li Z, Huang Y, Zhou C, Lin J, Wang Y, Cui F *et al.* Phytosomes loaded with Mitomycin C–Soybean Phosphatidylcholine Complex Developed for Drug Delivery. *Mol Pharmaceutics* 2013; 10:90-101.
- Xu K, Liu B, Ma Y. Physicochemical properties and antioxidant activities of luteolin-phospholipid complex. *Molecules* 2009; 14(9):3486-3493.
- Juanjuan L, Gan H. Advances in studies of phospholipids as carriers in skin topical application. *Journal of Nanjing Medical University.* 2007; 21(6):349-353.
- Matsui MS, Hsia A, Miller JD, Hanneman K, Scull H, Cooper KD *et al.* Non-sunscreen photoprotection: antioxidants add value to a sunscreen. *J Investig. Dermatol. Symp. Proc.* 2009; 14(1):56-59.