

# Development of Green Coffee Beans Extract Loaded Anti-aging Liposomal Gel

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## ABSTRACT

**Background:** Green beans of *Coffea arabica* L. Family: *Rubiaceae* are rich source of polyphenols that prevents the oxidative damage to the skin. In this study, Liposomes were formulated as drug carrier to enhance the skin permeability of polyphenols for topical administration. **Materials and Methods:** Extracts of green coffee beans were prepared by varying the ratios of methanol and water. Phytochemical analysis and *in vitro* antioxidant evaluation was conducted on all the extracts to choose the best extract for further studies. The cytotoxicity potential (MTT assay) and anti-elastase activity of chosen extract was evaluated on L929 cell lines using flow cytometer. The liposomes of the extract was prepared by thin film hydration method and optimized by varying the phosphatidylcholine to cholesterol ratio and gradually increasing the amount of extract. Liposomal formulation was converted into gel using carbopol<sup>®</sup> 974P as gelling agent. The prepared liposomal suspension and liposomal gel was evaluated for various formulation parameters. **Results:** The results of phytochemical analysis, *in vitro* antioxidant assays and *in vitro* evaluation on L929 cell lines concluded that 25% methanolic extract was nontoxic with highest phenolic content and exhibited good antielastase activity. The encapsulation efficiency of chlorogenic acid in the optimized liposomal formulation was 75%. The liposomal gel showed sustained release of the drug upto 12 hr compared to 6-7 hr of conventional formulation. **Conclusion:** The formulated green coffee bean extract loaded liposomal based gel may serve as potential carrier for antiaging effects.

**Key words:** Green coffee beans (GCB), Chlorogenic acid, Extract, Liposomes, Antioxidant, Antiaging.

## INTRODUCTION

The skin plays vital role in modulating body temperature, perceiving pain and pressure and acts as a crucial barrier against environmental insults and pollution making skin aging very evident.<sup>1</sup> Long term skin exposure to ultraviolet radiation produces free radicals which induces oxidative stress on skin leading to development of photoaging, sunburn, melanogenesis, immunosuppression and photocarcinogenesis.<sup>2</sup> Free radicals are extremely reactive oxygen molecules that are engaged in forming cross linkages with collagen molecules leading to loss of skin elasticity.<sup>3</sup> Aging manifest itself with sagging, thinning, dryness and spotting on the skin. Thus, with a desire of looking

young, the antiaging products are in great demand. Antioxidants from herbal origin have gained importance in cosmetic industry. Antioxidants help in repairing and preventing oxidative damage caused by free radicals. The antioxidant activity of herbs is majorly due to the redox properties of phenolic compounds.<sup>4</sup> Thus, antioxidants are found to be promising in delaying the signs of aging such as wrinkles, age spots and fine lines.

The green coffee (*Coffea arabica* L, *Rubiaceae*) beans are rich source of polyphenols such as chlorogenic acid and its related compounds such as caffeic acid, coumaric acid and ferulic acid that prevents the skin from oxidative damage.

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Studies have revealed *C. arabica* extracts are found to possess antibacterial,<sup>5</sup> antiviral,<sup>6</sup> anti-inflammatory,<sup>7</sup> skin wound healing,<sup>8</sup> reduction of oxidative damage to macromolecules<sup>9</sup> and suppressive activity of metalloproteinase expression.<sup>10</sup>

Chlorogenic acid, the major polyphenolic compound of green coffee beans has antioxidant and depigmenting property<sup>11</sup> and it inhibits UV-induced skin damage.<sup>12</sup> According to the scientific studies, green coffee beans contains a higher amount of chlorogenic acid than roasted coffee beans and other plants.<sup>13</sup>

The delivery of polyphenols through the skin is ineffective due to its limited penetration. Thus the formulation base or penetration enhancers that enhance its penetration is required.<sup>14</sup> So liposomes were selected as drug carrier as they are amphiphilic in nature and hydrophilic molecules can easily be embedded in the concentric bilayers. Liposomes being physiologically similar to the cell membrane are nontoxic in nature. The presence of phospholipids in the liposomes improves topical absorption of the drug from epidermis into the lower layers due to increased adhesion of the drug phospholipid complex on the skin.<sup>15</sup>

The aim of the present study is to develop a gel containing green coffee bean extract loaded liposomes to be used as antioxidant cosmetic formulation for antiaging effects.

## MATERIALS AND METHODS

### Materials

Green *coffee arabica* L. beans were obtained from the local market of Mumbai in August 2019 and was authenticated by Dr. Harshad M. Pandit. Ex-Head and Associate Professor of Botany, Guru Nanak Khalsa College, Mumbai, India. Phosphatidyl choline was purchased from HiMedia Laboratories Pvt Ltd, Cholesterol 99% was procured from Loba Chemie Pvt Ltd, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Chlorogenic acid was purchased from Otto chemie Pvt Ltd, Mumbai. Gallic acid and Ascorbic acid were procured from SD Fine Chemicals Ltd, Mumbai. All chemicals used in this study were of A. R. grade. Liposomes were produced using Rotary evaporator, Rotaeva, Equitron and UV-Visible Spectrophotometer, Shimadzu 1800 was used for evaluation of liposomes.

### Methods

#### Extraction Methodology

The powdered green coffee beans were defatted using petroleum ether 40-60°C (1:6 % w/v) for 3hr in a soxhlet apparatus. The defatted green coffee powder

was extracted with different solvents such as water, 25% methanol, 50% methanol, 75% methanol and 100% methanol for 2hr using a soxhlet system. The resulting extracts were concentrated in a porcelain dish on electric water bath at 80°C to remove the solvent. The prepared extracts were used for qualitative and quantitative analysis.

#### Total Phenolic Content

Total phenolic content was determined by Folin-Ciocalteu reagent method using standard procedure. Gallic acid was used as a standard and total polyphenolic content was expressed as mg/g gallic acid equivalent (GAE) from the calibration curve of gallic acid.<sup>16</sup>

#### Spectral Overlay

UV spectrometric method was used to determine the presence of chlorogenic acid in the extract. For preparation of standard solutions, 1mg/ml of standard chlorogenic acid was dissolved in phosphate buffer and further dilution were prepared to get 10 µg/ml of standard solution.

For sample solution, 25% of 1 mg/ml methanolic extract was dissolved in phosphate buffer and further dilution were prepared to get 10 µg/ml of standard solution.

An overlay spectra of both the solution was obtained using UV-Vis spectrophotometry.

#### Quantification of Chlorogenic acid in each extract Standard solution preparation

The standard chlorogenic acid was dissolved in the solvents such as water, 25% methanol, 50% methanol, 75% methanol and 100% methanol. In order to reduce the error in preparation procedure, the chlorogenic acid 10 ppm solution was prepared in each above solvent and then corresponding dilution ranging from concentration 2 to 10 ppm was prepared. The linearity plot was made by recording the absorbance at 324 nm using UV-Vis spectrophotometer.

#### Sample solution preparation

For Sample solution preparation, the extracts were dissolved in their respective solvents and 10 ppm solution was made. The absorbance for sample solution was recorded at 324 nm using UV-Vis spectrophotometer. The obtained absorbance was extrapolated in the linearity plot to find the concentration of chlorogenic acid in each extract.

## Determination of Antioxidant Activities

### DPPH (2,2-diphenyl -1-picrylhydrazyl) antioxidant assay

Radical scavenging activity of the extracts on DPPH radicals was investigated by following standard method.<sup>17</sup> Ascorbic acid was used as standard. The experiment was performed in triplicate. The percentage of DPPH radical scavenging activity was plotted against each extract concentration and IC<sub>50</sub> was determined.

### Nitric Oxide antioxidant assay

Nitric oxide radical scavenging activity was investigated by use of Griess Illosvoy reaction.<sup>18</sup> Ascorbic acid was used as standard. The experiment was performed in triplicate. The percentage of nitric oxide radical scavenging activity was plotted against each extract concentration and IC<sub>50</sub> was determined.

### Percentage Inhibition was for both the antioxidant studies was calculated using below mentioned formula

Scavenging activity was expressed as the inhibition percentage calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

### Determination of Cell viability by MTT Assay

Green coffee beans extract viz 25% methanolic extract was screened for cytotoxicity study against the L929 cell lines. 200 µl of cell suspension was seeded in 96-well plate with cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 24 hr. An appropriate concentrations of the test agent (25% methanolic extract) was added, the plate was incubated for 24 hr at 37°C in a 5% CO<sub>2</sub> atmosphere and the spent media was removed. MTT reagent was added to a final concentration of (0.5mg/mL) of total volume and the plates were incubated for 2-3 hr in dark condition. 100 µl of DMSO was added after removal of the MTT reagent and shaken gently. The absorbance was measured at 570nm and 630nm using a spectrophotometer on an ELISA reader. The IC<sub>50</sub> value was determined by using linear regression equation.

Formulae used for the study:

$$\% \text{ Cell Viability} = \frac{\text{Mean Absorbance of Sample Bank}}{\text{Mean Absorbance of Untreated} - \text{Blank}} \times 100$$

### Determination of Anti Elastase activity

The cells were cultured at the density of 3 × 10<sup>5</sup> cells/2 ml and incubated in a CO<sub>2</sub> incubator for 24 hr at 37°C.

**Table 1: Details of test compounds with different concentrations to the respective cell lines used for the study. GC bean represents Green coffee bean extract.**

Sr.No	Test Compound	Cell Line	Concentrations treated to cells
1.	Untreated	L929	No treatment
2.	Std (Camptothecin)	L929	11µM
3.	Blank	–	Only media without cells
4.	GC bean extract	L929	5 (12.5,25, 50,100,200 µg/ml)

For anti-elastase study, the cells were treated with 250uM epigallocatechin gallate (EGCG) were used as positive control, the cells treated with 200ug/ml of 25% of methanolic extract was used as test sample (Table 6) and the cells only with culture media were used as negative control and was incubated in 2ml of culture medium for 24 hr. The tubes were centrifuged for five minutes at 300 x g at 25°C and the cells were washed with PBS. 0.5 ml of BD Cytotfix/Cytoperm solution was added after removal of PBS and tubes were kept undisturbed for 10 min. 0.5% of bovine serum albumin (BSA) in PBS and 0.1% sodium azide was used to wash the cells. 20 µL of Mouse Anti-Human Neutrophil Elastase/ ELA2 Alexa Fluor® 647-conjugated monoclonal antibody was added, mixed and incubated for 30 min at room temperature under dark condition. The cells were treated with 0.1% sodium azide and 0.5 mL of PBS and analyzed by Flow Cytometry with the excitation and emission of 650nm and 668nm respectively.

In this study, test compound namely Green coffee bean extract (25% methanolic extract) and standard Epigallocatechin gallate (EGCG) were used to evaluate its effect on ELA-2 (conjugated mouse anti-human elastase antibody) expression in L929 cell lines.

### Preparation of green coffee beans extract loaded liposomes

The thin film hydration method as described by Bangham *et al.*<sup>19</sup> was used to formulate liposomes. The lipid mixture was prepared by dissolving phosphatidylcholine and cholesterol in chloroform and methanol (2:1) in round bottom flask (RBF) and glass beads were added for homogeneous film formation. The RBF was attached to rotary flash evaporator (Roteva, equitron) and allowed to rotate at 80 rpm/min in a thermostated water bath at 40°C. The organic solvents were removed by slow application of vacuum leading to homogenous lipid film formation

**Table 2: Optimization of liposomal batches based on drug:lipid ratio and PC:CH ratio.**

Batch no	Ratio of PC: CH	Extract (mg)	Globule size (microns)	Chlorogenic acid encapsulated (%)
B1	1:0	10	2-5	61.52
B2	1:0.1	10	2.5-5.5	70.5
B3	1:0.2	10	1.5-5	72.8
B4	1:0.5	10	2-4	62.00
B5	1:1	10	1.5-5	68.00
B6	1:0.1	20	1-3	63.2
B7	1:0.2	20	0.5-5	74.8
B8	1:0.5	20	2-4	54.7
B9	1:1	20	1.5-5	68.42
B10	1:0.1	30	2.5-5.5	71.33
B11	1:0.2	30	2-5	52.34
B12	1:0.5	30	0.5-5	56.50
B13	1:1	30	1.5-3.5	58.65

PC-Phosphotidylcholine, CH-Cholesterol

on the walls of the flask. The film was allowed to dry for 1h for complete removal of organic solvents.

The dried lipid film was hydrated with phosphate buffer (pH: 5.4) containing dissolved extract (25% of methanolic extract) and then the RBF was rotated at 150 rpm/min in a thermostated water bath maintained at 46°C that is above the transition temperature of lipid. Rotation was continued for 60 min until homogeneous suspension was obtained. The liposomes produced using this method are large and heterogenous in size, thus bath sonication method was used for 30 min to downsize the liposomes. The green coffee beans extract based liposomal suspension was allowed to stand for 2 hr at room temperature to ensure complete hydration. The liposomal suspension was stored in amber coloured glass bottle in refrigerator until further use.

### Characterization of liposomes

The following are the parameters for which the prepared liposomes were characterized

### Determination of Encapsulation Efficiency

UV-Vis spectrophotometer was used to estimate the encapsulation efficiency of chlorogenic acid in green coffee bean extract loaded liposomes. The absorbance of chlorogenic acid remaining in the supernatant after centrifugation was measured at 324 nm using UV-Vis spectrophotometer. Then, the concentration was calculated from calibration plot obtained for standard chlorogenic acid.

Encapsulation efficiency was calculated as follows

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Total amount of drug added} - \text{Amount of drug detected in supernatant}}{\text{Total amount of drug added}} \times 100$$

### Total amount of drug added

### Optical Microscopy

A drop of liposomal suspension was placed on a clean glass slide and observed under the power of 45X and 100X of the optical microscope (Motic microscope).

### Determination of Vesicle size, Polydispersibility Index (PDI) and Zeta Potential

Vesicle size and Polydispersibility Index were analysed by using dynamic light scattering instrument with a computerized inspection system (Malvern particle size analyzer). Freshly prepared liposomal batches were diluted in the ratio of 1:100 with the deionized water. All measurements were done in triplicate at 25 ±0.5°C. Liposomal suspension was diluted with the deionized water and zeta potential was determined using Malvern zetasizer.

### Transmission Electron Microscopy (TEM)

TEM analysis was carried out for optimized drug loaded suspension by transmission electron microscopy (Tecnai T20, 200Kev, FEI). A drop of suspension was placed with the aid of micropipette on the grid. The grid was dried well. The dried grid containing the sample was bombarded with electrons accelerated at 200 KV. Then vesicle size and liposomal morphology was visualized by TEM under vacuum (Figure 1).

### Preparation of Liposomal Gel

Green coffee beans extract loaded liposomal gel was prepared by using carbopol®974P gel base. Accurately 0.5% of carbopol®974P was weighed and kept for hydration in double distilled water containing

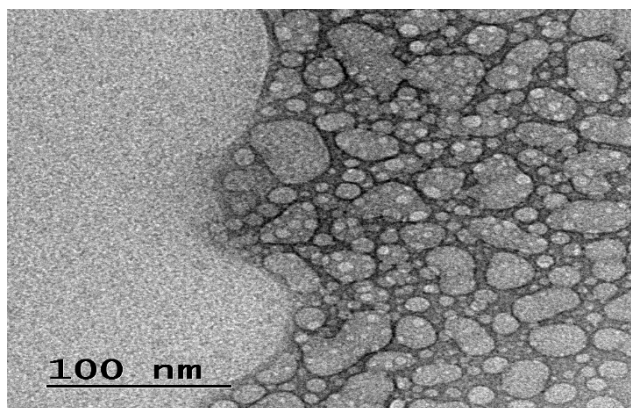


Figure 1: TEM image of green coffee beans loaded liposomes.



preservatives overnight. Liposomal pellets obtained after centrifugation was dispersed in distilled water by sonication and added to hydrated carbopol solution with stirring. Gelling was induced by neutralization using triethanolamine (TEA). The gel was evaluated for colour, texture (smoothness and greasiness), pH, viscosity, drug content, spreadability and *in vitro* release drug study.

### In vitro drug release study

The Franz diffusion cells apparatus having receptor compartment capacity of 22 ml and surface area of 3.91 cm<sup>2</sup> was used to estimate *in vitro* drug release. Dialysis membrane (molecular weight cut-off 12,000-14,000) which was pre-hydrated by soaking in buffer overnight were mounted on the cells. Phosphate buffer pH 5.4 (37°C ± 0.5°C) was used as receptor fluid. Reservoir solution was stirred at 100 rpm/min using a magnetic bead. 0.5 g of gel was placed in donor compartment on the membrane. Aliquots were collected at 1, 2, 3, 4, 5, 6,7,8,12 and 24 hr intervals and analyzed by UV-Vis spectrophotometer at 324nm.<sup>20</sup>

## RESULTS AND DISCUSSION

### Extraction Methodology

The percentage yields of green *coffee arabica L.* beans extracts is presented in the Table 3. The physical appearance of all the extracts were dark yellow and dark brown in colour. The 25% of methanolic extract showed the highest percentage yield as 26.54 ± 0.13 compared to all other extracts.

### Total Phenolic Content

The Folin-Ciocalteu method was used to estimate the total phenolic content of all the extracts. The TPC

**Table 3: Consolidated data of % yield, Total phenolic content and mg/g of chlorogenic acid in each extract. The values in the table was obtained by calculating the mean ± SD (n=3). GAE represents Gallic acid equivalent.**

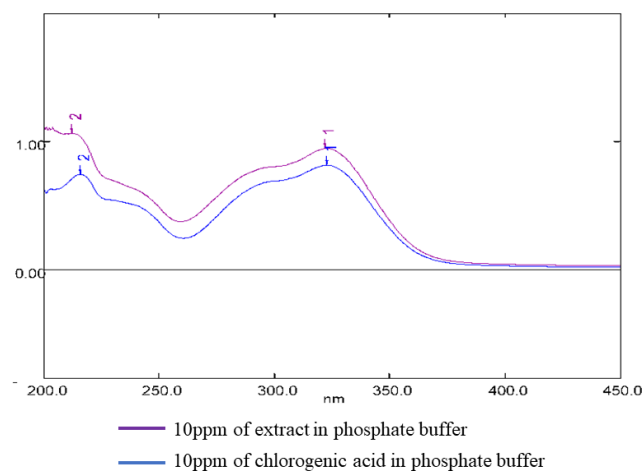
Extracts	% Yield	TPC (mg/g of GAE)	Chlorogenic acid content (mg/g)
Aqueous extract	24.42 ± 0.103	478 ± 0.34	37.1 ± 0.24
Methanolic extract	22.26 ± 0.203	530 ± 0.96	41.2 ± 0.35
25 % methanolic extract	26.54 ± 0.13	580 ± 1.52	43.4 ± 1.25
50 % methanolic extract	23.02 ± 0.23	456 ± 0.13	40.2 ± 0.31
75 % methanolic extract	25.30 ± 0.31	460 ± 1.15	38.9 ± 1.54

of all the extract is expressed in terms of Gallic acid equivalent (The standard curve equation:  $y = 0.0031 + 0.3477x$ ,  $r^2 = 0.9953$ ) mentioned in the Table 1. Amongst all the extracts major phenolic content is estimated in 25% of methanolic extract.

### Spectral Overlay

#### Quantification of Chlorogenic acid in each extract

The amount of chlorogenic acid content present in each extract is mentioned in Table 3 and Figure 2. As per the results the highest amount chlorogenic acid was found to be present in 25% of methanolic extract.



**Figure 2: Spectral overlay of standard chlorogenic acid and presence of chlorogenic acid in extract. From Figure 2 (Spectral overlay) confirmed that the 25% methanolic extract had presence of chlorogenic acid in it.**

### Determination of Antioxidant Activities

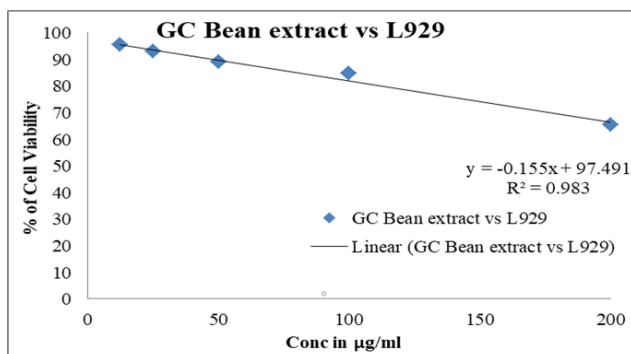
The extracts exhibited antioxidant activity in concentration-dependent manner. The antioxidant activity of the extract was expressed in terms of IC<sub>50</sub>. Consolidated results of DPPH assay and Nitric oxide antioxidant assay is mentioned in Table 4.

**Table 4: Consolidated data of antioxidant assays of various extracts of green coffee beans.**

Extracts	IC <sub>50</sub> (µg/ml) DPPH assay	IC <sub>50</sub> (µg/ml) Nitric oxide assay
Ascorbic acid (Standard)	55.41 ± 0.12	54.70 ± 0.08
Aqueous extract	230.89 ± 0.25	234.75 ± 0.12
Methanolic extract	146.52 ± 0.44	148.34 ± 0.24
25% methanolic extract	143.92 ± 0.28	145.23 ± 0.18
50% methanolic extract	184.62 ± 0.20	186.25 ± 0.13
75% methanolic extract	172.43 ± 0.22	177.62 ± 0.45

**Table 5: Represents IC<sub>50</sub> concentration of green coffee bean extract and the standard camptothecin against L929 cell lines.**

Sr. no	Test compounds	IC <sub>50</sub> (µg/ml)
1.	Camptothecin	23
2.	GC bean extract	306.38



**Graph 1: Scatter graph showing the % cell viability of L929 cell line against green coffee bean extract and the inhibitory concentration (IC<sub>50</sub> value) observed is 306.38 µg/ml.**

Based on the results obtained from total phenolic content, quantification of chlorogenic acid and antioxidant assay 25% methanolic extract was found to contain highest phenolic content, greater quantity of chlorogenic acid and maximal radical scavenging activity compared to other extracts.

#### Determination of Cell viability by MTT Assay

Viability assay is an assay that determines the ability of tissues, cells or organs to maintain or recover the state of survival. Cytotoxicity study of the test compound, green coffee bean extract against L929 cell lines in statistical data of cell cytotoxicity study by ELISA reader suggested us that green coffee bean extract showed moderate cytotoxicity potential properties with Inhibitory concentration (IC<sub>50</sub>) at 306.38 µg/ml compared to standard drug Camptothecin with 23µg/ml Table 5. The observation strongly suggest us that the green coffee bean extract do not have significant cytotoxicity potential with the concentration ranging from 12.5-200 µg/ml respectively with incubation period of 24 hrs at 37°C (Graph 1).

#### Determination of Anti Elastase activity

The enzyme elastase is capable of breaking down elastin, an insoluble elastic fibrous protein that together with collagen contributes to the mechanical strength of the connective tissue. Several studies have suggested that both skin aging and anti-wrinkle effects

significantly corresponds to reduced elastase activity. Thus antielastase studies was carried out to confirm the utility of green coffee bean extract as a anti-aging agent. In this study, test compound namely Green coffee bean extract and Std, EGCG were used to evaluate its effect on ELA-2 (conjugated mouse anti-human Elastase antibody) expression in L929 cell line. The concentrations of the compounds used in the experiment were as follows:

The given test compound GC bean extract suppressed the ELA-2 expression in L929 cell line. The relative mean fluorescence intensity values of ELA-2 were almost similarly decreased in std, Epigallocatechin gallate and test compound green coffee bean extract. (Table 7; Figure 3 and Histogram 1). Hence, GC bean can be considered as a good anti elastase agent.

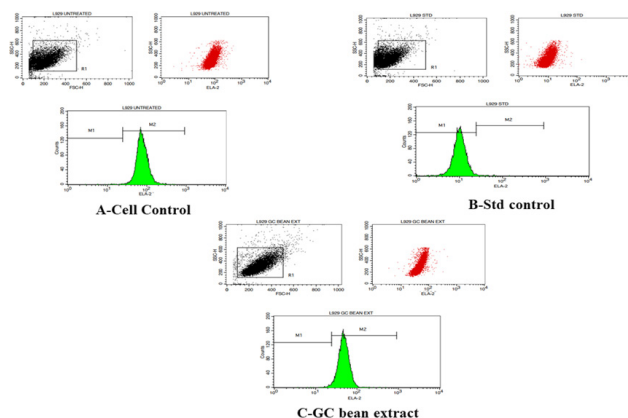
#### Preparation of green coffee beans extract loaded liposomes

In this study thin film hydration method was used to formulate liposomes. The reason for selecting this

**Table 6: Represents the concentration of green coffee bean extract and standard compound used to determine antiaging potential against L929 cell lines.**

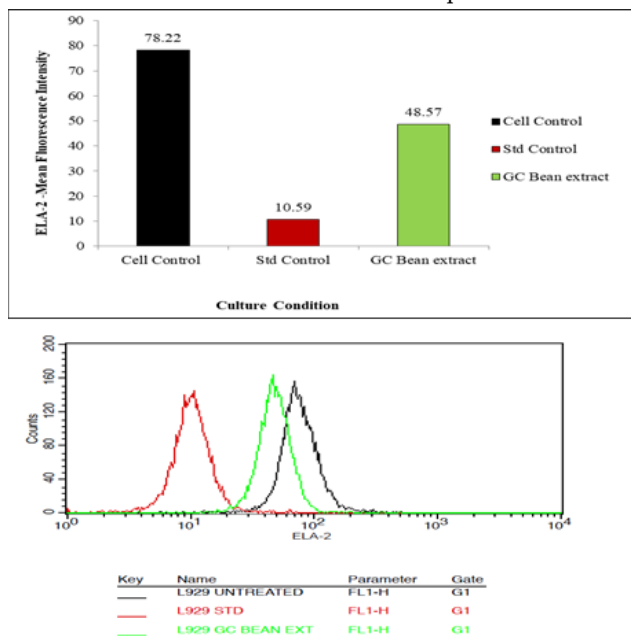
Sr.No	Test Compounds	Cell Line	Concentrations
1	Cell Control/Untreated	L929	No treatment
2	STD (EGCG)	L929	250uM
3	GC bean extract	L929	200ug/ml

Effect of the GC bean extract on ELA-2 expression in L929 cell line



**Figure 3: Histograms depicting the ELA-2 expression observed in cell control (A), Std Control (B) and GC bean extract treated L929 cell line with the incubation period of 24hrs (C). Analysis was done by using BD FACS Calibur, Cell Quest Pro software (Version: 6.0). Histograms of the gated L929 singlets distinguish cells at the M1 and M2 phases. (Here M1 refers to Positive expression/region and M2 refers to the Negative expression/region).**

Relative mean fluorescence intensity values of ELA-2 in L929 cell line treated with test compound GC bean



**Histogram 1: Overlay histogram of GC bean extract.**

**Table 7: Showing the mean fluorescence intensity values of ELA-2 against the untreated, std and drug treated L929 Cells (GC bean extract).**

Parameters	ELA-2 Relative Mean Fluorescence Intensity
Cell Control	78.22
Std Control	10.59
Green Coffee Bean Extract	48.57

method is as it is simplest method for preparing multilamellar vesicles and is capable of loading higher mass of hydrophilic molecule such as chlorogenic acid compared to unilamellar vesicles. Thin film hydration method was found to give 75% of encapsulation efficiency for chlorogenic acid present in the extract Table 2. Thus, this method was used for preparation of the liposomes.

### Characterization of liposomes

#### Optical Microscopy

Multilamellar vesicle was clearly observed (Figure 4). Microscopic observation were used to evaluate the shape and size of the vesicles was noted for all the prepared batches.

### Determination of Polydispersibility Index (PDI), Zeta Potential and Vesicle size

PDI basically appears for size distribution of the vesicles in a given sample. The numerical value of PDI starts with 0.0 (perfectly uniform particle size) to 1.1 (highly polydisperse particle size). In case of lipid based carrier systems such as liposomes and nanoliposomes a size of 0.3 and below is acceptable as it indicates homogeneous population of phospholipid vesicles.<sup>21</sup>

Zeta potential is a significant characterization technique which is employed to understand physical stability of the particles. A value from -30 mV to +30mV is generally considered to have sufficient repulsive force to attain better physical colloidal stability.<sup>22</sup>

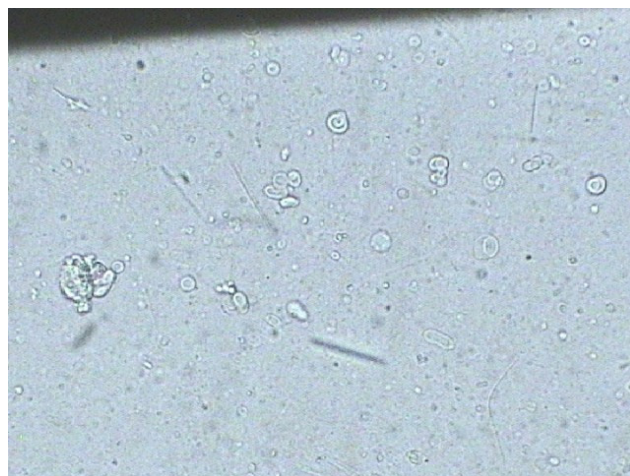
The thin film hydration method gives large and heterogenous multilamellar vesicles. Size analysis of the produced liposomes revealed z- average (d.nm) size of 864.2 nm, PDI of 0.375 and Zeta Potential of -12.4 with good entrapment efficiency.

### Transmission Electron Microscopy (TEM)

Morphological analysis of these liposomes by TEM showed spherical shaped liposomes.

### Preparation of Liposomal Gel

Liposomal dispersion was simply mixed with the polymer to give corresponding gels. Liposomal gel formulation is advantageous as the fusion of the vesicles can effectively be avoided or minimized as the polymer serves as spacer between the liposomes. Thereby, the vesicle size is not affected because it is a controlled polymer/liposome interaction process. Liposomal based cream formulation was not chosen as stability issues are reported in emulsion-based products due to



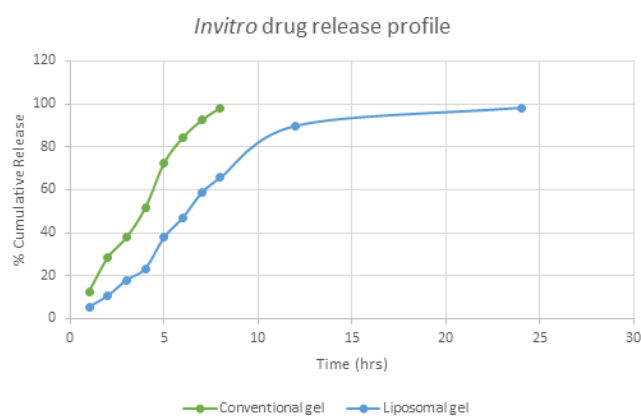
**Figure 4: Shows large multilamellar vesicles visible under 100X magnification.**

presence of surfactant and excess oil which may interact with vesicles. Various properties such as viscosity can easily be controlled by varying the concentration of the polymer or changing the polymer type. Thus liposomal gels were prepared as they circumvent the stability issue, provide controlled release, easy to prepare and have aesthetic appeal as skin care cosmetics.<sup>23</sup>

The prepared lipogels were pale yellow in color and opaque. 0.5% w/w of carbopol®974P was found have good consistency and smooth appearance devoid of any aggregation. The drug content for the liposomal gel was found to be  $94.16 \pm 1.3$  with viscosity of gel in the range of 13,500 cps to 16,500 cps and spreadability around 7-8 g.cm/s.

### In vitro drug release profile

The outermost layer of the skin is stratum corneum and is mainly composed of protein keratin and lipids. The intercellular lipids plays a vital role in controlling the percutaneous absorption. The intercellular lipids may interact with the phospholipids present in the liposomes and thereby cause swelling of the lipids without altering the multiple bilayer structure of the stratum corneum. These swollen lipids causes drug to accumulate and forms an intracutaneous depot. Although the mechanism of topically applied liposomes is not fully understood, but lipid composition, surface charge and liposomal lamellarity primarily plays an important role in drug disposition. Studies has also revealed topical delivery is also influenced by size of liposomes.<sup>24</sup> The *in vitro* drug release was performed using dialysis membrane. The *in vitro* =release study of liposomal gel has shown sustained release effect upto 12 hr compared to 7-8 hr of conventional gel Graph 2.



**Graph 2: Comparative *in vitro* drug release through dialysis membrane.**

## CONCLUSION

Green *Coffea arabica* beans extracts were screened for total phenolic content, quantification of chlorogenic acid and antioxidant assay. Based on the results obtained from these studies 25% of methanolic extract was chosen to be incorporated into liposomes as it had shown highest phenolic content, greater quantity of chlorogenic acid and better antioxidant activity. Before incorporating the extract into the liposomes it was screened for MTT and antielastase assay. Based on MTT assay the extract had no cell cytotoxicity potential and as per antielastase assay the extract had antielastase activity. Thus the extract can be utilized to formulate antiaging liposomes. The multilamellar vesicles were prepared using thin film hydration method. The prepared liposomes had good encapsulation efficiency and better physical stability. The extract based liposomal suspension was formulated into liposomal gel. As per *in vitro* drug release study the liposomal gel has shown prolonged release effect upto 12 hr compared to the conventional gel. Hence green coffee beans extract loaded liposomes can be considered to be an effective carrier for the topical delivery with antiaging potential.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS:

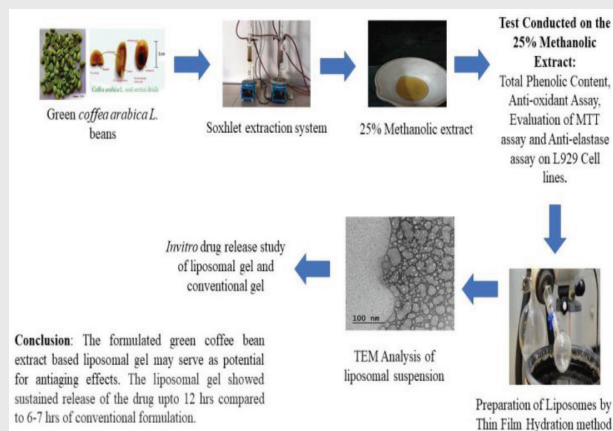
**TPC:** Total phenolic content; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **GAE:** Gallic acid equivalent; **GC:** Green Coffee; **GCB:** Green coffee beans; **ELA-2 (Conjugated mouse anti-human Elastase antibody):** Neutrophil Elastase; **EGCG:** Epigallocatechin gallate; **PC:** Phosphatidylcholine; **CH:** Cholesterol; **RBF:** Round Bottom Flask; **TEM:** Transmission Electron Microscopy; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **PDI:** Polydispersibility Index; **IC<sub>50</sub>:** Half-Maximal Inhibitory Concentration.



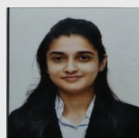
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## PICTORIAL ABSTRACT



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## SUMMARY

Aging of skin is an inevitable process. The skin aging is characterized by loss of elasticity, wrinkling, laxity, sagging and rough texture of the skin. The process of aging causes structural and functional changes in the cutaneous cells as well as extracellular matrix components such as elastin and collagens. One cannot stop the aging process but, obviously, one can delay the sign's of aging by application of anti-aging agents. The topical route of drug delivery is well known in the field of pharamaceutics. Over the centuries, the topical drug delivery system has been investigated to deliver diverse range of therapeutic agents. However, the stratum corneum of the skin sets the rate limiting barrier for the transport of drugs via topical route. To overcome these challenges, liposomes were selected as drug carrier as they are ampiphilic in nature and hydrophilic molecules can easily be embedded in the concentric bilayers. Liposomes being physiologically similar to the cell membrane they are nontoxic in nature. The phospholipids present in liposomes allows increased adhesion of the complex of bioactive compound to the skin surface and improves topical absorption from epidermis into the deepest layers of the skin. In the Present study, the developed green *coffee arabica L.* beans based liposomal gel may be used as antioxidant cosmetic formulation for antiaging effects.

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