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Liposomal delivery of Jamun extract in gel formulation and its evaluation

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Abstract

Jamun is a flavorful fruit rich in antioxidant compounds. A fruit possessing Antidiabetic properties. The fruits of ***Jamun*** were collected and authenticated, the pulp was dried and extracted with alcohol and distilled water. The extractive values were found using ethanol and distilled water. The pharmacognostic evaluation parameters of ***Jamun*** pulp powder were estimated, including extractive values, total ash value, acid-insoluble ash value, water soluble ash value and loss on drying, foaming index, and swelling index. The phytochemical screening of ***Jamun*** extract was performed and it was found to contain a variety of phytochemicals, including anthocyanins, vitamins, polyphenols, sugars. The preformulation studies were conducted for the excipients added to the formulation. Liposomes enclosing ***Jamun*** extract were formulated. Liposomes were formulated to protect the skin against damaging conditions in the external environment. They have the ability to provide protection for a longer duration of time and providing a clear-cut advantage over traditional dosage forms. The liposomes formulated were added to Carbopol 940 base to form a liposomal gel formulation. The formulation was evaluated for organoleptic properties, pH, spreadability, homogeneity, viscosity, UV analysis, IR Spectrum interpretation, Topographic imaging, percentage release characteristics, and stability studies.

Keywords: Liposomal gel, Jamun pulp, Carbopol 940, Pharmacognostical evaluation, Phytochemical evaluation.

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1. Introduction

Regarding phytochemicals, the Greek word for plants is "Phyto." Phytochemicals come in a wide range of families and support the human body in different ways. Plant compounds with protective and disease-preventive abilities are called phytochemicals. The various phytoconstituents include Glycosides, Tannins, Saponins, Resins, Oleo-resins, Terpenoids, Alkaloids, related nitrogen compounds, and phenolic constituents [1]. The fruit is violet,enclosed in a purple fruit covering while the seeds are brown, and they have a sweet and astringent taste.The fruit pulp has fragrance and is oval in shape, enclosing a single seed[2]. Liposomes are small microscopic vesicles of round shape that can be made from cholesterol and phospholipids. Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shells consisting of lipids arranged in a bilayer, unilamellar or a concentric series of multiple bilayers (multilamellar bilayers). The major advantage of liposomal drug delivery systems is that they provide as a carrier for hydrophilic and lipophilic molecules and provide controlled drug delivery for various therapeutic purposes. Liposomal properties vary with lipid ratio, surface charge, size and the technique for formulation [3]. Liposomes because of the layering property, biocompatibility, and selectivity are used as a medication transporter [4]. Liposomes are helpful because they can deliver a number of medications with potential therapeutic

effects or other qualities. Liposomes are colloidal carriers with diameters ranging from 0.01 to 5.0 μm. Because the drug must first be released from the liposome before it can be metabolized and excreted, drugs encapsulated in liposomes maintain their therapeutic level for an extended duration of time.These are tiny spherical vesicles that can be made from naturalphospholipids and cholesterol[4].

Fig 1: Jamun fruits and Jamun pulp powder

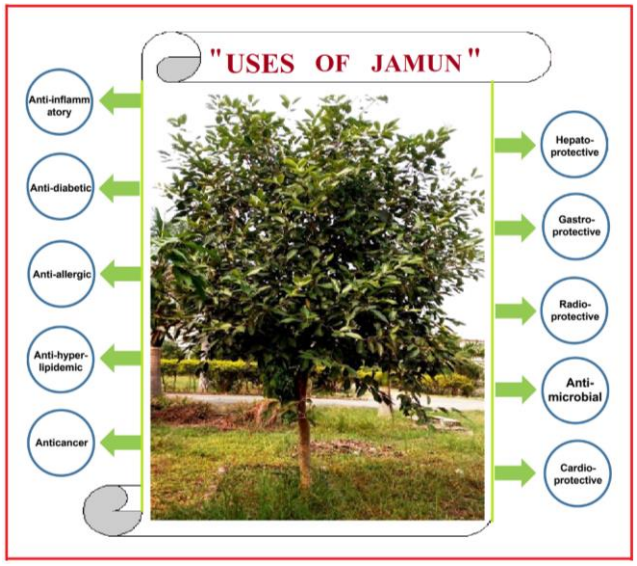


Fig 2: Jamun of use

2. Materials and methods

2.1 Botanical identification

Jamun was collected in Sidhari, Azamgarh, Uttar Pradesh, India, in April 2025. The botanical identification was carried out by Dr. N.K. Dubey, a botanist with ACME Research Solutions in New Delhi, India. The reference number for the specimen was ACME/PA/11074.

2.2 Materials

Cholesterol, Triethanolamine, Ethanol, Chloroform, Acetic acid, were purchased from local market. The extraction was carried out by using dried ***Jamun*** pulp powder.

2.3 Phytochemical Screening Tests:

2.3.1 Saponin detection using the foam test:

Extracts were diluted with 20 milliliters of distilled water and shaken in a graduated cylinder for 15 minutes. When a 1 cm layer of foam formed, saponins are present.

2.3.2 Test for amino acids (Tyrosine):

A few drops of Millon's reagent were added to 2ml of the extract. The presence of amino acids was indicated by their dark red appearance.

2.3.3 Test for glycosides:

2.3.3.1Borntrager's test for Anthraquinone glycosides:

Added dilute sulphuric acid to 3 ml of the extract solution. Boiled and filtered. Added 3ml of chloroform to the cold filtrate. Shaken and added the organic solvent, separated the organic solvent. On addition of 1 ml of diluted ammonia, it turned rose pink.

2.3.3.2 Cardiac glycosides detection using the legal test:

Added 1 ml of pyridine and 1 ml of sodium nitroprusside to 2 ml of extract solution. The color turned pink.

2.3.4 Test for Tannins and phenolic compounds:

2.3.4.1 5% $FeCl_3$ solution:

Added 5% ferric chloride solution to 2ml of extract solution. A deep blue-black coloured precipitate appeared.

2.3.4.2 Bromine water:

Mixed two milliliters of extract solution with a few drops of bromine water. Bromine-containing water became decolorized.

2.3.4.3 Dilute iodine solution:

Added a few drops of dilute solution of iodine to 2 ml of extract solution. A brown colour appeared.

2.5 Sugar Reduction Test:

2 ml of Fehling's solution A and Fehling's solution B reagents were mixed, added to the extract and heated to a gentle boil. Reducing sugars, if present, indicated a brick-red precipitate formed at the bottom of the test tube.

2.6 Test for alkaloids (Hager's):

2 ml of HCl was mixed with 5 ml of extract. 1ml of Hager's reagent was added to the sample solution. A yellow-coloured precipitate indicated the presence of Alkaloids.

2.7 Phytosterol detection using Salkowski's test:

Chloroform treatment and filtration were applied to the extracts. A few drops of concentrated sulphuric acid were added to the filtrate and allowed to stand, shaken, a golden yellow appearance indicated the presence of triterpenes.

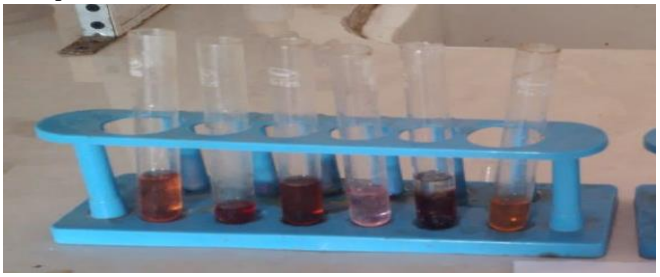


Fig. 3: Jamun pulp phytochemical testing

2.4 Physical Characterization Parameters:

2.4.1 Determination of loss on drying:

2.4.1.1 Gravimetric methods:

In glass Petri dishes that had been previously weighed, 1.5g of pulp was dried for three hours at 100°C in a hot air oven. After cooling to room temperature, the Petri dishes were kept in a desiccator and their weight was recorded. Until the final weight stabilized, the drying process was continued. The moisture content was expressed as a percentage of the weight of pulp and the difference in weight was observed.

$$\textbf{Moisture content } \% = \frac{w1 - w2}{w1} \times 100$$

2.4.2 Determination of Total Ash Value:

2 grams of powder was placed in a silica crucible and heated gradually to 450°C in a muffle furnace until carbon-free ash was produced. After cooling in a desiccator, the ash was weighed. The value of total ash was calculated as milligrams per gram of air-dried material.

2.4.3 Determination of acid insoluble ash:

The ash that was produced in total ash was gently boiled with 25 milliliters of hydrochloric acid for 5 minutes. An ashless filter paper was used to filter the mixture, and hot water was used to wash insoluble material until the filtrate was neutral. After being placed in a crucible, the filter paper containing the insoluble material was ignited to a constant weight. After cooling in a desiccator, the residue was weighed. The amount of acid-insoluble ash was calculated in milligrams per gram of air-dried material.

2.4.4 Determination of Water- soluble ash:

Boiled the ash that was produced in total ash with 25 milliliters of water for 5 minutes. An ashless filter paper was used to collect the insoluble material. After being cleaned with hot water, it was ignited for fifteen minutes in a crucible. The weight of total ash was deducted from the weight of insoluble matter. In milligrams per gram of air-dried material, the amount of water-soluble ash was determined.

2.4.5 Fluorescence analysis:

The ***Jamun*** leaves, stem-bark and fruit powder was treated with various chemical reagents and placed in UV Cabinet. The powder of leaves, stem and fruits were prepared after passing it through mesh 40. The fluorescence characters were studied both in daylight and in UV light (254 and 366 nm) using different solvents like sulphuric acid, hydrochloric acid, ferric chloride acetic acid etc.

2.4.6 Different solvent extractive value:

This technique uses various solvents to extract the active ingredients from a weighed amount of *Jamun* pulp powder. Chloroform, acetic acid, methanol, and water were used as solvents for determination of extractive values. 2 grams of ***Jamun*** pulp powder, was transferred to a stoppered conical flask. After that, 50 ml of a solvent was added, shaken for six hours, and left to stand for eighteen hours. The solution was filtered, dried in a Petri dish and weighed. The solvent extractive value % was then calculated.

Fig 4: Extracts in different solvents.

2.5 Preparation of liposome by rotary flash evaporator method:

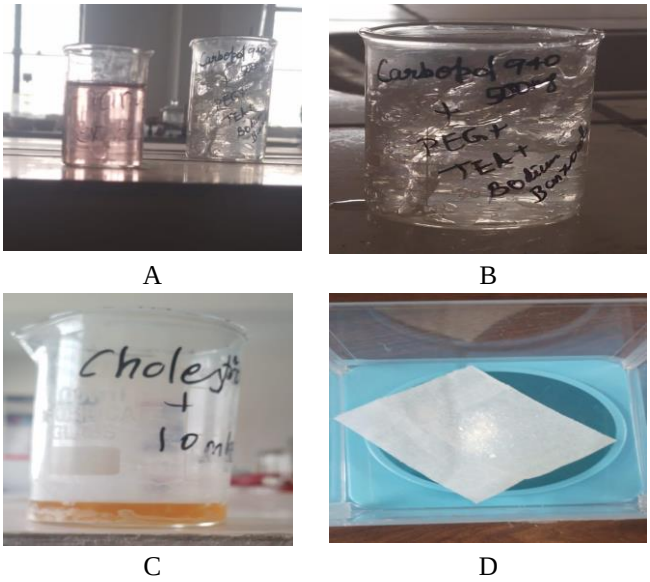
Liposomes were prepared by rotary flash evaporator method. Ten milliliters of ethanol was used as a solvent to dissolve 100 mg of cholesterol. A 500 ml round bottom flask was filled with the extract. In a thermostatically controlled water bath at 40 °C with a vacuum of 240 mmHg, the flask was rotated in a rotary flash evaporator at 40 rpm for 20 minutes. This process formed a very thin layer of dry lipids on the flask and removed the solvent gradually. Ten milliliters of saline phosphate buffer (pH 7.4) containing *Jamun* pulp extract was used to gradually hydrate the dry lipid film. The rotatory flask was left at room temperature for two hours and rotated at the same speed. Full lipid hydration was achieved by leaving the liposome overnight at 4 °C.

2.6 Preparation of liposomal gel:

Carbopol 940 was weighed and added gradually to a phosphate buffer solution (pH 5.8) while being continuously stirred with a paddle stirrer. Following the addition of solid material, the gel was given at least 12 hours to swell while being stirred moderately or until completely swollen and clear. To formulate a uniform gel dispersion, additional ingredients were added, including 15% w/v polyethylene glycol-400 (PEG-400), triethanolamine (0.5% w/v), and sodium benzoate (0.5% w/v) to the buffer used to prepare the gel. The liposomal dispersions and gels were combined in a 1:5 (w/w) ratio (liposome dispersion/gel) to formulate liposomal gel formulations [5].

Table 1: Ingredients in Liposomal gel formulation:

S No.	Ingredients	Quantity
1	Liposomes containing Jamun extract	100 mg
2	Triethanolamine	q.s.
3	Carbopol 940	1.5
4	Sodium benzoate	q.s.
5	Polyethylene glycol-400(PEG-400)	2 ml



A B C D

Fig 5: To prepare the liposomal gel incorporating *Jamun* extract

(A) Jamun pulp extract
(B) To prepare the Carbopol gel.
(C) To prepare the cholesterol solution.
(D) Weight of Cholesterol.

2.7 Liposome Evaluation: -

2.7.1 Vesicle size determination:

Vesicle size was determined using TEM.

2.7.2 Transmission electron microscopy (TEM):

To assess surface morphology, a drop of the sample was put on a copper grid coated with carbon. After 15 minutes, it was stained with a 1% phosphotungustic acid aqueous solution. After drying the grid completely, samples were examined using a transmission electron microscope [11].

2.7.3 Calibration curve in phosphate buffer pH 7.4:

A stock solution of ***Jamun*** pulp was diluted to prepare a standard drug solution with concentrations of 10, 20, 30, 40, and 50 µg/ml. Absorbances were recorded against a blank PBS, and a calibration curve was plotted [9].

2.8 Evaluation of prepared gels:

2.8.1 Physical evaluation:

The physical evaluation involved the gel's colour, odour, texture, and stability [6].

2.8.2 Measurement of pH

The pH meter was calibrated with the help of a standard buffer solution. Weighed 0.5 gm of gel and dissolved in 50.0ml of distilled water, pH was measured with the help of a digital pH meter [7].

2.8.3 Removal:

The ease of removal of the liposomal gel applied was determined by washing the applied part with tap water.

2.8.4 Homogeneity:

The formulations were tested for the homogeneity by visual appearance and by touch [10].

2.8.5 Spreadability:

The spreadability of gel was determined using a weighed amount of prepared gel and applied on to the skin with slow speed, it was spread on to the skin and observed the ease with which the gel was spread and whether it left a residue after washing with water for 10 seconds [8].

2.8.6 Viscosity study:

Viscosity was determined by using a Brookfield viscometer, with 30 gm of gel placed in a 50 ml beaker. The readings were measured after five minutes, and the viscosity was calculated using factor. The procedure was repeated three times.[4]

2.8.7 Stability study:

The stability of liposomes was assessed at various temperatures, revealing a gradual decline in entrapment efficiency. The most significant loss of entrapped drug occurred at 37°C, while the least occurred at 4°C. The study suggested that coalescence within liposomal suspension may cause drug leaching [9].

3. Results And Discussion

3.1 Powder macroscopy:

Color: The presence of anthocyanin pigments gives ***jamun*** pulp powder a deep purple colour.
Texture: The powder usually has a fine, free-flowing texture.
Odour - Characteristic.
Taste - Sweet and Sour.

3.2 Physical Characteristics and Extractive values:

The various physical characteristics and extractive values for ***Jamun*** pulp are mentioned in Table 3 & 4. The effects of various chemical agents on powdered ***Jamun*** pulp are mentioned in Table 2.

Table 2: Phytochemical Screening of *Jamun* pulp powder

S. No.	Phytochemicals	Methanol	Chloroform	Water	Acetic acid
1	Alkaloids	+	+	+	-
2	Glycoside	+	+	+	+
3	Tannin & Phenolic	+	-	+	+
4	Flavonoids	+	+	+	+
5	Reducing sugar	+	+	+	+
6	Amino acid	-	-	-	+
7	Steroid	+	+	-	+
8	Anthraquinone	+	+	+	+
9	Saponin	+	-	+	-

Table 3: Extractive values of *Jamun* pulp powder with different solvents

S No.	Solvent	Extractive values (% w/w)	Colour
1	**Methanol**	4	Baby pink
2	**Chloroform**	0.108	Very light Baby pink
3	**Water**	2.412	Baby pink
4	**Acetic acid**	1.16	Red

Table 4: Physical characteristics of powdered *Jamun* pulp powder:

S. No.	Parameters	Observation
1	Swelling Index	0.4
2	Foaming Index	0.31
3	Loss on drying	1.042
6	Total ash value	1.05
7	Acid insoluble ash	0.7
8	Water soluble ash	0.98

3.3 Fluorescence analysis of powder:

The ***Jamun*** pulp powder was treated with various chemicals and observed under day light and fluorescence light (UV light 245 nm), the results are mentioned in Table 7.

Table 5: Fluorescence analysis of *Jamun* pulp powder:

S. No.	Reagent with powder	UV Light	Visible Light
1	Pulp Powder+ Water	Yellowish green	Light green
2	Pulp Powder + Ethanol	Dark green	Brown
3	Pulp Powder +Methanol	Light green	Brown
4	Pulp Powder+NaOH	Dark green	Brown
5	Pulp Powder +HCl	Yellowish green	Light green
6	Pulp Powder +H2SO4	Dark green	Brown
7	Pulp Powder +KOH	Dark green	Yellowish green
8	Pulp Powder+HNO3	Green	Light green
9	Pulp Powder + Acetic acid	Yellowish green	Light green

3.4 Physical evaluation of liposomal gel:

When formulation was kept for a long time, it was observed that there was no change in organoleptic properties of liposomal gel.

Table 6: Physical evaluation of liposomal gel

S. No.	Specification	Limits
1	State	Semi-solid
2	Colour	Pale white
3	Odor	Characteristic
4	Texture	Smooth

3.5 Spreadability:

The gel was applied onto skin and the extent to which the gel easily spread was determined.

3.6 Measurement of pH:

The pH of the formulated gel was determined using a digital pH meter. The electrode was immersed in the gel and readings were recorded with the help of pH meter. (Table 7)

Table 7: pH of the formulated gel

Sr. No.	Formulation	pH
1	F1	7.4

3.7 Viscosity determination:

Viscosity determination was done using Brookfield viscometer using suitable spindle number and rpm.

3.8 Jamun pulp calibration curve in PBS (pH 7.4):

The calibration curve was plotted between absorbance and concentration. The correlation coefficient for ***Jamun*** pulp in PBS (pH 7.4) was found out to be 0.999 and 0.998, respectively. In the concentration range of 1–5 µg/ml, the estimation method for ***Jamun*** pulp powder demonstrated a linear relationship.

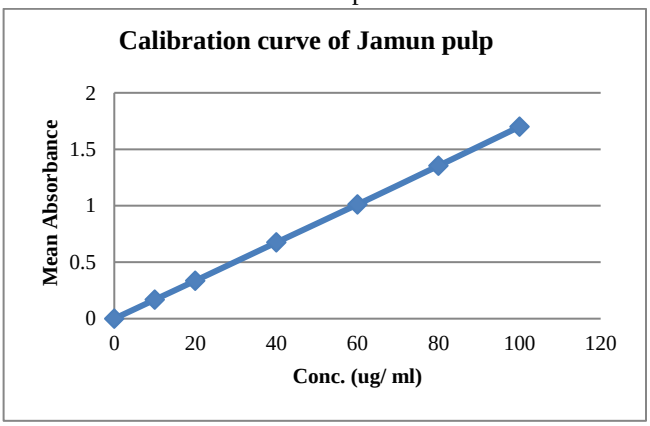


Fig 6: Calibration curve of Jamun pulp

3.9 Transmission Electron Microscopy:

Liposomes, tiny, spherical vesicles, were identified by transmission electron microscopy (TEM). Variation in size distribution, possibly due to charge neutralization, ranged from 100 to 200 nm.

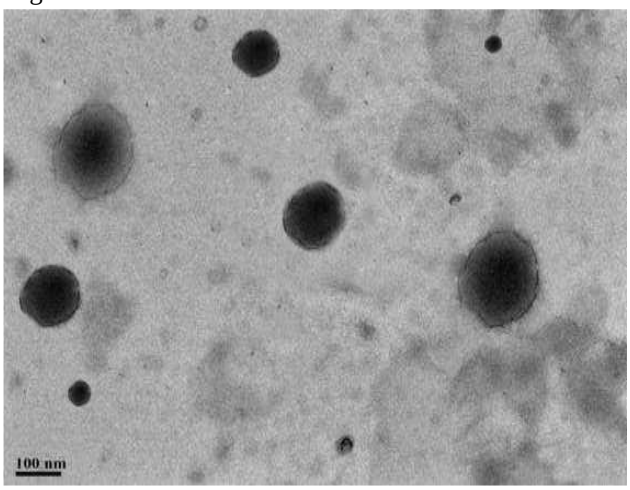


Fig 7: TEM image of liposomes

3.10 Vesicle size determination:

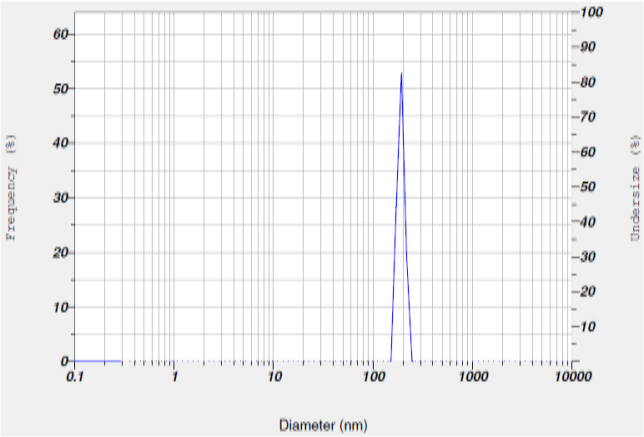


Fig 8: Graph of Vesicle size.

3.11 FT-IR study: The FTIR spectra obtained for ***Jamun*** pulp is shown in figure below.

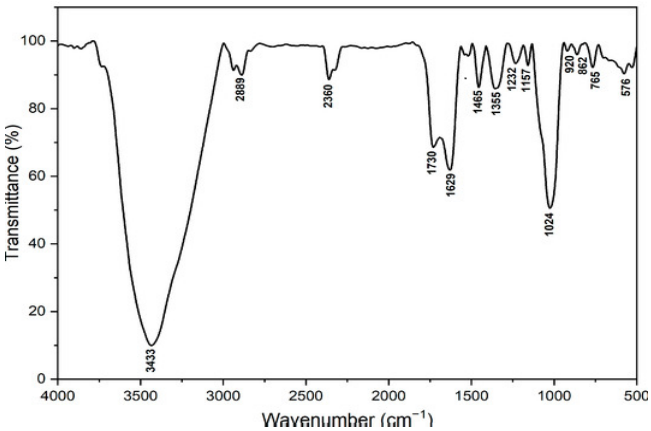


Fig 9: Major peaks in the FTIR spectrum of Jamun pulp

3.12 Viscosity:

The viscosity of gel was in between 500-1000 cps which indicated that the liposomal gel was easily spreadable. The formulated liposomal gel indicated good spreadable property.

3.13 Type of smear:

After application of liposomal gel, the type of smear formed on the skin were non greasy.

3.14 Homogeneity:

Formulation produced a uniform distribution of extracts in liposomal gel. This was confirmed by visual appearance and by touch.

3.15 After feel:

Emolliency was good. The amount of residue left after the application of fixed amount of liposomal gel was found negligible.

3.16 Removal:

The formulated liposomal gel applied on the skin was easily removed by washing with tap water.

3.17 Appearance:

When formulation was kept for a long time, it was found that there was no change in organoleptic properties of liposomal gel as shown in Table below

Table 8: Physical Characteristics of liposomal gel.

S. No.	Specification	Limits
1	State	Semi-solid
2	Colour	White
3	Odor	Characteristic
4	Texture	Smooth

3.18 Stability study:

The stability of liposomes was assessed at various temperatures, revealing a gradual decline in entrapment efficiency. The most significant loss of entrapped occurred at 37°C, while the least occurred at 4°C. The study suggested that coalescence within liposomal gel may cause drug leaching.

Table 9: Effect of temperature on percentage entrapment of *Jamun* pulp in liposomes on storage for 28 days

Time (Days)	Log entrapment percent		
	4^0C	25^0C	37^0C
0	**1.857**	**1.857**	**1.843**
7	**1.854**	**1.829**	**1.798**
14	**1.855**	**1.789**	**1.764**
21	**1.853**	**1.771**	**1.721**
Slightly 28	**1.855**	**1.764**	**1.699**
Degradation Constant (k) / day	**0.0001**	**0.0061**	**0.0091**

4. Conclusion

The surface of skin has a complicated layered structure. The skin is composed of bilipid layer. The drug has to pass through the various layers of skin to penetrate inside the skin and reach the vascular system. The liposomes are an intricate system of medication incorporating Jamun extract within a liposomal covering imparting the gel a long-lasting effect on the skin.

In India, ***Jamun*** (*Syzgium cumini L.*) is a fruit that is enjoyed by all socioeconomic groups. It is easily accessible and also has therapeutic qualities. ***Jamun*** contains a variety of phytoconstituents and nutritional constituents, these properties are important for health concerns and useful for human being. In this study ***Jamun*** fruit pulp was analyzed by standard analytical methods. These studies focused an understanding of the values of mineral and chemical composition of ***jamun*** fruit as it has a lot of beneficial uses, effects and further research on it is to be done. The formulation F1 was developed by using Carbopol 940 polymer for gel formulation. Liposomal gel incorporating extract of *Jamun pulp* were evaluated for the

physiochemical parameters such as drug content, pH of formulation, viscosity, spreadability, *in vitro* drug diffusion using Franz diffusion cell. Viscosity measurement revealed that formulation was appropriate in viscosity for gel formulation.

Jamun fruits bearing violet hue, sweetish and sour in taste. A fruit bearing anthocyanins, full of antioxidants, Anti- diabetic in nature was selected for the research study.

The plant was authenticated and was given a specimen number. The fruit pulp powder was evaluated for Pharmacognostical and Physico-chemical standardization parameters and reported. The pulp powder was extracted with alcohol and distilled water as solvents. The extractive values were reported. The preliminary Phytochemical Screening of Jamun pulp powder extracts were performed and represented in Tabular form. The IR Spectrum study of ***Jamun*** pulp powder was done and reported. The major functional groups were found to be alcohol, phenyl groups. The drug entrapment studies and percentage release studies, stability studies were also evaluated.

The Jamun pulp powder extract was formulated into liposomal form which was mixed into Carbopol 940 as base formulating liposomal gel. The liposomal gel was evaluated for spreadability, homogeneity, vesicle size, zeta potential determination, Topographic imaging, viscosity etc. The formulated liposomal gel was found to be easily spreadable, soothing and producing a glowing effect on the surface of skin, enhancing its appearance and
vibrant radiant looks.

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