Formulation and Characterization of Nanoparticles loaded Drug Delivery System Using Natural Polymers for treatment of Inflammation

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ABSTRACT

The objective of research work was formulation and Characterization of Nanoparticles loaded Drug Delivery System Using Natural Polymers for treatment of Inflammation. The prepared plant extract were standardized by using pharmacognostic and phytochemical study. In pharmacognostic and phytochemical study a number of parameters used such as ash values, extractive values, soluble and insoluble extractive values and tests for alkaloid, flavonois, saponin, steroinds aglycosides etc. The stanadardized plant extract was used for the development of nanoparticles using Tween-80 and polyethanol glycol. The developed nanoparticles were characterized using particle size, drug loading, viscosity and pH analysis. The prepared plant extract loaded nanoparticles were used for the formulation of topical gel. The results of evaluation paramneters indicated that optimized formulation was suitable and best for human use. The findings of the current report account for the potential use of Carissa carandas loaded formulation nanoparticles act as an effective delivery system for encapsulation of water insoluble basic drugs, e.g., MFA in a magnetized carrier

Key words: Nanoparticles, pharmacognostic, phytochemical, particle size, drug loading, viscosity, pH.

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Introduction

Topical drug delivery is gaining great attention in the field of pharmaceutical industry [8]. Skin is the largest organ of human body and stratum corneum, and the outer most layer of skin acts as main barrier against the entry of substances into the body. Different techniques were implied to enhance the skin permeability of drugs/active pharmaceutical ingredients. One of the approaches is to encapsulate the drug into nanoparticles, which increases the drug permeation through the skin [10]. Chitosan is widely used polymer for nanoparticles. Chitosan is a naturally derived polymer having biocompatibility, biodegradability, mucoadhesivity, and permeation enhancement activity (Bhosale et al., 2020, Arif et al., 2016, Singh et al., 2015).

Carissa carandas, commonly known as Karanda or Bengal currant, is a shrub belonging to the Apocynaceae family. It is native to India and is widely distributed in various parts of Asia and Africa. *Carissa carandas* is known for its edible fruits and has been used in traditional medicine for its medicinal properties. *Carissa carandas* contains various phytochemical compounds, including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and glycosides. These bioactive compounds contribute to the plant's medicinal properties and antioxidant activity. Medicinal Purposes: Various parts of *Carissa carandas*, including the roots, leaves, and fruits, have been traditionally used in Ayurveda and other traditional medicinal systems to treat various ailments. It is believed to possess anti-inflammatory, analgesic, anti-diarrheal, and anti-microbial properties. Culinary Uses: The ripe fruits of Carissa carandas are consumed raw or used in the preparation of jams, jellies, sauces, and desserts. They have a tangy flavor and can be eaten fresh or used in culinary recipes (Bhosale et al., 2020, Arif et al., 2016, Singh et al., 2015, Hameed et al., 2021).

MATERIALS AND METHODS:

Collection and identification of plant material

The Carissa carandas were collected from nearby market. The plant materials were collected during the months of November 2022. The plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.

Pharmacognostic evaluation (Bajaj et al., 2012, Gupta et al., 2015, Bhati et al., 2014, Mourya et al., 2017, Tiwari et al., 2016

Air-dried powdered material was subjected to qualitative and quantitative physicochemical estimations. The procedures were followed as mentioned earlier.

Physical evaluation

The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined.

Ash values

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analysts favour mixing of sulphuric acid with the powdered crude drug before ashing and this sulphated ash value is normally less fusible than ordinary ash.

% ASH = ((ashed wt.) - (crucible wt.)) x 100/((crucible and sample wt.) - (crucible wt.))

Determination of total ash value

Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

The formula for calculating the total ash value is:

Total Ash (%) = (Weight of Ash / Initial Sample Weight) x 100

Determination of acid insoluble ash value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Acid-Insoluble Ash (%) = (Weight of Acid-Insoluble Ash / Initial Sample Weight) x 100

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Water-Soluble Ash (%) = (Weight of Water-Soluble Ash / Initial Sample Weight) x 100

Loss on drying

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven). About 1.5 gm. of powdered drug was weighed accurately in a tared porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

Extractive values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value

5gms of the air-dried coarse powder of the plant material was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the airdried drug.

Determination of water soluble extractive value

Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Preparation of extracts

Suitable amount of powdered drug was packed in soxhlet apparatus and extracted with different polarity of solvent.

Preparation of the extracts

Extraction was carried out in Soxhlet apparatus not exceeding 600 c and the extract thus obtained was concentrated below 60^0c.

Soxhlet Extraction

The method of hot continuous percolation was chosen for extraction of Carissa carandas following reasons: Obtain dried and ground *Carissa carandas* plant material, weighted a suitable amount of the powdered material, placed the material inside a sample thimble, set up the Soxhlet extraction apparatus, added a suitable organic solvent to the round-bottom flask, heated the flask to start the extraction process and then after allow the extraction to continue for several hours or overnight. Remove the flask containing the solvent and extracted compounds. Evaporated the solvent to obtain a concentrated extract and Analyzed or purify the extract as needed.

Figure 1: Soxhlet Extraction

Preliminary Phytochemical Screening of extract

Phytochemicals (from Greek phyto, meaning "plant") are chemicals produced by plants having an important role to protect and defence plant. They are regarded as a research compounds to establish the proof of their possible health effects. The different qualitative chemical tests were performed for establishing profile of a given extract for its chemical composition.

Preparation of test solution

1.5 gram of extract was accurately measured. Test solution was prepared by dissolving extract separately. It was prepared as per the need, labelled properly and stored in a refrigerator when not required. Each extract was tested for the presence of different phyto-constituents qualitatively and quantitatively. Alkaloids, Carbohydrates, steroids, glycosides, phenols, flavonoids, saponins, triterpenoids, Phlobatanins, cardiac glucosides, Anthraquinone, proteins were tested by usual prescribe methods.

Detection of alkaloids:

These atr nitrogeneous organic compounds present in the plant. They have marked ans strong physiological action on humans.

Mayer's test

Preparation of reagents

Mayer's reagent was prepared by dissolving 1.35 gram of Mercuric chloride in 60 ml of distilled water. 5 grams of Potassium iodide was dissolved in a 10 ml of distilled water. Both the solutions were prepared separately till all solute was completely dissolved. The solutions were mixed slowly and final volume of 100 ml was achieved in a volumetric flask using distilled water.

Procedure

To a 0.5 ml of test solutions of Unprocessed Turmeric and OT 3 ml of Mayer's reagent was added alongside of the test tube. Appearance of milky white precipitate suggests positive result that is alkaloids are present.

Wagner's Test

Preparation of reagent:

Wagner's reagent was prepared by mixing 1.27 gram of Iodine and 2 gram of potassium iodide in a 20 ml D.W. Solution was prepared and diluted to 100 ml in a volumetric flask using D.W.

Procedure:

To a 0.5 ml of test solutions of Unprocessed Turmeric and PT 3 drops of Wagner's reagent was added alongside of the test tube. Appearance of Reddish brown precipitate indicates positive result that is alkaloids are present.

Hager's Test

Preparation of reagent: Hager's reagent consists of saturated aqueous solution of picric acid and was prepared.

Procedure:

To a 0.5 ml of test solutions 1 ml of Hager's reagent was added. The prominent reddish- yellow colour was developed which indicates the presence of Alkaloids.

Detection of Glycosides

Glycosides are also derived from plant and generally formed by the replacement of (OH) hydroxyl group in the sugar molecule.

Molish's Test

Preparation of the reagent

Molish's Reagent was prepared by dissolving 1 mg of Alpha- Naphtol in 2 ml of alcohol and immediately used for estimation.

Procedure

To a few ml of test solutions Molish's reagent was added mix and slowly few drops of concentrated sulphuric acid was added without disturbing and along the side of a test tube. Blue violet ring was appearing at a junction of two liquid. This indicates presence of Glycosides in the both extracts.

Benedict 's test

Preparation of the reagent:

Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water Copper sulphate (17.3 g) dissolved in 100 ml distilled water was added to it. The final volume was made 1000ml using D.W.

Procedure:

To a 1 ml of test solutions 0.5 ml Benedict's reagent was added mixed and kept in boiling water bath for 20 minutes. Appearance of yellowish-brown colour indicates presence of glycoside /reducing sugar

Fehling Test

Preparation of the reagent: Fehling's solution

Copper sulphate (34.66 g) was dissolved in 100ml distilled water and using D.W. final volume of 500ml was made. Fehling's solution B: potassium sodium tartrate (173 g) and sodium hydroxide (50 g) was dissolved in 200 ml of D.W. The final volume was made up to 500ml.

Procedure

To a 1 ml of test solutions 0.2 ml of Fehling's A and 0.2 ml of Fehling's B solution was added, mixed and kept in boiling water bath for 20 minutes. Appearance of yellowish-brown colour indicates presence of glycoside /reducing sugar.

Detection of Cardiac glycosides

Considerable number of plants scattered throughout the plant kingdom contain C21or C-24 steoidal glycosides which exert a slowing and strengthing effects on the heart failing (Wc, Evan16 th edition 2004).

4.8.3.1 Preparation of the test solution as follows.

For cardiac glycosides the 50 mg of both extracts was hydrolysed with 10 ml concentrated hydrochloric acid for two hours on a water bath. The solution was filtered and filtrate was used for Keller-killani test and Borntrager's test.

Keller-killiani test for deoxy sugars

Preparation of reagent

5gram of Ferric chloride is dissolved in 100 ml D.W.

Procedure

Deoxy-sugars are found in cardiac glycosides. 2ml of aqueous solutions of both extracts was taken. Approximately 0.1 ml of glacial acetic acid, one drop of Ferric chloride solution and 1 drop of concentrated sulphuric acid was added. At the junction of the liquids a reddish-brown colour is produced which gradually becomes blue.

Detection of Anthraquinone glycosides

These are aromatic compounds where two keto gropus are attached to benzene ring occure in plant as glycosides.

Borntrager's test

Procedure

To 2 ml of test solution filtrate 2 ml of chloroform was added and shaken. Chloroform layer was separated. 1 ml of ammonium solution was added. Pink colour was developed. It indicates presence of Anthraquinone glycosides.

Detection of Saponin glycosides

These are plant origine glycosides.Some glycosides are known as saponins (Latin sttpo.soap) which are characterized by their property of producing a loathing aqueous solution.

Foam Test:

Procedure

Approximately 50mg of both extracts were taken and dissolved in 25 ml D.W. It was transformed into two graduated cylinders. Both are shaken for 15 minutes. A foam layer was appearing which indicate presence of Saponins.

Detection of Flavonoids:

The flavonoids are all structurally derived from the parent substance flavone. Flavonoids are generally present in plants bound to sugar as glycosides (Harborne 1 st edition 2005), and occur both in the Free State and as glycosides. More than 2000 of these compounds are now known, with nearly 500 occurring in the Free State. They are formed from three acetate units and a phenyl propane unit.

Alkaline reagent test:

Preparation of the reagent

10% ammonium hydroxide solution was prepared.

Procedure:

To the 1ml of aqueous solution of both extract 0.5 ml 10% ammonium hydroxide solution was added. The yellow colour appears indicate the presence of flavanoids

Shinoda Test (cyanidin test):

Preparation of test solution

50 mg of both extracts were dissolved in methanol separately and used for this test.

Procedure

1 ml of the test solution was taken and three drops of concentrated hydrochloric acid were added. Then the magnesium turnings were put into thesolution and observed. Positive test indicates the appearance of pink red colour.

Detection of Proteins

Proteins are made up of amino acids linked by peptide bonds.

Biuret test:

Biuret test is specific for protein and peptide bond. Commercially available biuret reagent (Bio-Lab) was used.

Procedure

To a 1 ml of both extract solutions 0.5 ml of Biuret reagent was added. The solution was incubated for 5 minutes at 370C. Development of violet, purple colour indicates the presence of protein in the extracts.

Lowry Test

A. Preparation of reagent

1ml of a reagent (made by adding 1ml 0.5% CuS04.5H20 in 1% sodium citrate to 50ml 2% Na2C03 in 0.1 M NaOH) and the solution was left to stand for 10min at room temperature.

Procedure

Aqueous solutions of both extracts were added in two different tubes followed by freshly prepared reagent. It was followed by the addition of 0.1ml of the Folin-ceiocalteu reagent (commercial reagent diluted with water to give a 1M acid solution). Appearance of dark purple colour indicates positive test.

Ninhydrin test

The reaction of Ninhydrin primary amino group form purple colour called Ruhemann's purple.(Mendel Friedman 2004) as it was discovered by Siegfried Rhuhemann in 1910. It is unique reaction, at pH 5.5 ninhydrin reacts with primary amines, proteins, peptides, and amino acids give

visible distinctive purple colour. The reaction is not affected by the yellow colour present in many foods, plant and tissue extracts.

Preparation of reagent

Freshly prepared Ninhydrin reagent was used. 10 mg of Ninhydrin was dissolved in 20 ml of acetone and kept in air tight brown bottle.

Procedure

3 ml of aqueous solutions of both extract was taken. To this 2 ml of Ninhydrin reagent was added and kept in boiling water bath for 20 minutes. Appearance of the purple bluish colour indicates the presence of proteins (Bajaj et al., 2012, Gupta et al., 2015, Bhati et al., 2014, Mourya et al., 2017, Tiwari et al., 2016).

Preparation of Nanoparticles formulation

Carissa carandas extract was obtained using an appropriate extraction method. The extract was then concentrated, and various formulation codes were prepared by altering the concentrations of Tween 80, PEG, and the ethanol-to-distilled water ratio. The formulation codes used in this study were FM-1, FM-2, FM-3, FM-4, and FM-5. Each formulation contained 10 mg of Carissa carandas extract. The effects of different stirring speeds (500, 1000, 1500 rpm) and homogenization cycle (3, 6, 9 cycles) were optimized based on the measurements of particle size and encapsulation efficiency, respectively. Nanoparticles were prepared using a nanoprecipitation method, and their particle size, encapsulation efficiency, and stability were characterized (Müller et al., 2002, Jain et al., 1997, Jong et al., 2008, Kumar et al., 2021).

Table 1: Formulation Table Nanoparticle

S.NO	Formulation Code	Tween 80	Polyethylene glycol	Ethanol:Distilled Water	Carissa carandas (mg)
1.	FM-1	2.5%	1.5	30:70	10
2.	FM-2	4.2%	1.25	45:55	10
3.	FM-3	5.5%	2.5	25:75	10
4.	FM-4	5.0%	1.25	15:85	10
5.	FM-5	4.5%	2.5	15:85	10

Physicochemical characterization of nanoparticles

Drug loading and loading efficiency (Müller et al., 2002, Jain et al., 1997, Jong et al., 2008)

The ratio of the amount of drug in the nanoparticle to the overall amount of drug used in their formation is known as drug loading efficiency. The ratio of the quantity of drug in a nanoparticle to the overall amount of drug used in its formulation is known as drug loading efficiency. To determine the quantity of drug entrapped in the experimental formulations, drug loading was done. 2 ml of a water-acetonitrile solution (40:60 v/v) were used to suspend the necessary amount of nanoparticles (2 mg). The mixture was vortexed for five minutes, then shaken for three to four hours at 37°C in an incubator shaker. After 5 minutes of 10,000 rpm centrifugation, the supernatant was collected. The difference between the absorbance of the nanoparticle formulation and the blank formulation was used to quantify the actual quantity of drug contained in nanoparticles. The following formulae were used to determine the proportion of real medication loading and loading effectiveness:

Percentage of loading = $\frac{Amount\ of\ in\ present\ drug\ nanoparticles}{Weight\ of\ nanopartic\ le\ analyzed}$ x 100

Percentage of efficiency = $\frac{Actual\ loading\ drug}{Theoretical\ \ drug\ loading}$ x 100

Yield Percentage

Regarding the total amount of raw materials utilised for the formulation, the quantity of nanoparticles produced was calculated. The lyophilized nanoparticles were weighed, and the formulations' % yield was determined using the formula below:

Percentage of loading = $\frac{Amount\ of\ nanopartic\ obtained}{Total\ of\ polymer\ and\ drug\ used}$ x 100

4.10 Particle size, size distribution and zeta potential

Particle size and size distribution are the most important characteristics to be evaluated for nanoparticles systems. The particle size distribution is reported as Poly Dispersity Index (PDI). The particle size and Polydispersity index (PDI) of herbal loaded polymeric nanosuspension are measured using a Zeta sizer nano ZS (Malvern Instruments Ltd., Malvern, UK). The samples are placed in the analyzer chamber and the readings are carried out at a 90° angle with respect to the incident beam. Disposable cuvettes of 0.75ml capacity are used for all measurements (Kumar et al., 2018).

Zeta potential

The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. The zeta potential of herbal loaded polymeric nanosuspension are measured using a Zeta sizer Nano ZS. A potential of ±150 mV is set in the instrument. Disposable cuvettes of 0.75ml capacity are used for all measurements (Müller et al., 2002, Jain et al., 1997, Jong et al., 2008).

Preparation of nanoparticle Gel

The gels were prepared by dispersion method using Polymer. Accurately weighed Propylene Glycol was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell it for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. The Nanoparticle concentrate (pellet) with drug equivalent to 1%w/w was incorporated into the priorly formed gel base. Triethanolamine was added to maintain the pH and for the spontaneous gel formation. To this gel solution optimized Nanoparticle dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-80C (Kumar et al., 2018, Kumar et al., 2021).

Table 2: Preparation of Nanoparticle Gel

S.NO	Formulation Code (Nanoparticle Gel - NG)	Propylene Glycol	Purified Water	Triethanolamine	Nanoparticle formulation
1.	NG-1	15	10	1.5	Nanoparticle formulation 1
2.	NG-2	10	25	3.5	Nanoparticle formulation 2
3.	NG-3	15	25	1.5	Nanoparticle formulation 3

4.	NG-4	10	20	3.5	Nanoparticle formulation 4
5.	NG-5	15	25	4.5	Nanoparticle formulation 5

Characterization & Evaluation of Formulation

Evaluation of Gel Formulation

All prepared formulations of gel were characterized for:

Physical Evaluation

Physical parameters such as color and appearance of the herbal gel were observed manually.

Measurement of pH

Skin pH is an important indicator of topical preparation stability especially in case of gels. The average pH of human skin ranges from 5.5 to 6. Hence, the pH of topical preparations must be in accordance with skin ís pH (7).The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average value was calculated.

Figure 2: Digital pH meter

Spreadibility

Spreadibility was determined by the apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped of from the edges. The top plate was then subjected to pull of 80 gms

weight with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadibility. Spreadibility was calculated using the formula given below:

$S = M \times L / T$

Where,

S = Spreadibility,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

Figure 3: Spreadibility of formulation

Consistency

The measurement of consistency of the prepared formulation was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance travelled by the cone was noted after 10sec.

Figure 4: Measurement of consistency of the prepared

Homogeneity

All the developed gels were tested for homogeneity by visual inspection after setting the gels in the container. They were observed for their appearance and presence of any aggregates.

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle No. 7 at 50 rpm at room temperature. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookefield Viscometer manual.

Figure 5: Measuring Viscosity of gel

Result & Discussion:

India is well known for it to be the house for traditional herbal medicines. Their presence well documented in Ayurveda, Siddha, and Unani. About 60% of the world's population is using the alternative system of medicines. The rural community eventually uses such medications for primary healthcare issues but also used by developed and developing countries wherever modern medicines do exist. Looking at the immense applications with less possible side effects, the whole world bent upon using herbals for medicinal use. World Health Organization has identified and listed 21,000 plants, which used for medicinal applications. Approximately 2500 species are of their indigenous to India, out of which 150 species have already been utilized commercially on a large scale. India is well known as the botanical garden of the world and considered as the largest exporter and importer of herbal drugs.

Physicochemical Standardization of Proposed Plant Drug

The powdered plant material of Carissa carandas was evaluated by standard procedure for the determination of following physicochemical parameters.

The plant Carissa carandas was identified collected and shade dried. The shade dried leaves were converted into moderately coarse powder. This powdered material was used for the determination of various physicochemical properties. Obtained values were recorded in the following table:

Table 3: Standardization parameters of *Carissa carandas*

S.No	Parameters % w/w	Ethanol drug ext
1.	Ash value	13.5
2.	Water soluble ash	14.56
3.	Acid insoluble ash	5.4

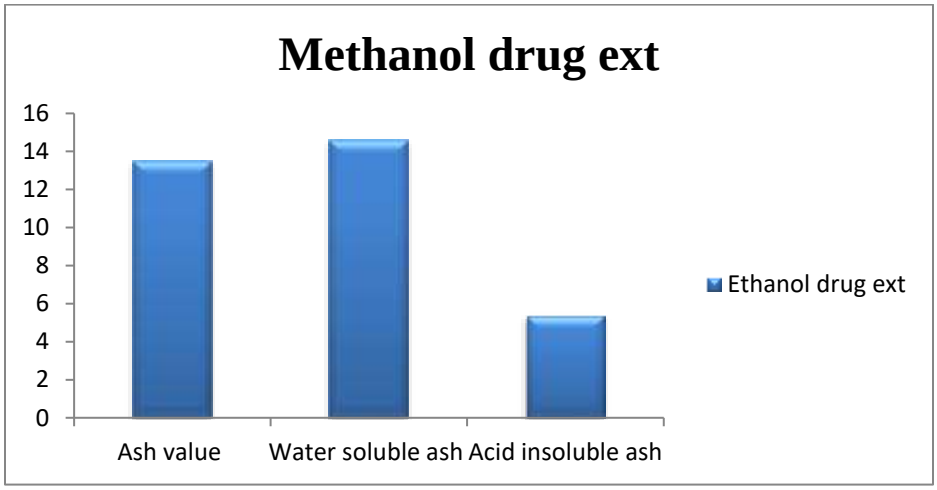

Figure 6: Graph of Standardization parameters of *Carissa carandas*

Extraction of Selected Plant Drug

The moderately coarse powder of the leaves of Carissa carandas was subjected to extraction with water as solvent in order to find the active constituents. The obtained aqueous extract of leaves of Carissa carandas was dried and weighed. The yield was found to be (10.50 % w/w of crude drug)

of Ethanolic extract with semisolid mass of Greenish when extracted with water for 18 hrs. Obtained results were recorded in table:

Table 4: Extractive values obtained from Carissa carandas

S.No	Solvent	Color of extract	% yield
1.	Ethanolic	Greenish	10.50 %

Phytochemical extraction

Phytochemical extraction is the process of obtaining and isolating specific bioactive compounds from plants. The table provided lists various Phyto-constituents and their presence or absence in the Ethanol ic extract of Carissa carandas. According to the table, the extract contains alkaloids, flavonoids, phenols, saponins, amino acids and proteins, glycosides, and carbohydrates. However, fixed oils and fats, as well as phytosterols, are absent in the extract. It is important to note that the presence of these phytoconstituents may vary depending on several factors, such as the plant part used and the extraction method employed. In the case of Carissa carandas, there are several methods for extracting its phytochemicals, including:

The table presents the presence or absence of various phytoconstituents in Ethanolic extract of Carissa carandas. The phytoconstituents included alkaloids, flavonoids, phenols, saponins, tannins, amino acids and proteins, fixed oils and fats, glycosides, phytosterols, and carbohydrates.

Preliminary phytochemical screening

Table 5: Preliminary phytochemical screening

S.No	Phytoconstituents	Carissa carandas Ethanolic extract
1	Alkaloids	+
2	Flavonoids	-
3	Phenols	-
4	Saponins	-
5	Tannins	+

6	Amino acids and Proteins	-
7	Fixed oils and Fats	-
8	Glycosides	+
9	Steroids	+
10	Carbohydrates	+

+" Present "-" Absent

Physicochemical characterization of nanoparticles

Understanding the physicochemical properties of nanomaterials and how functionalizations modify their surface, altering their properties, is fundamental to defining better strategies of use. Therefore, a good understanding of nanoparticle requires the use of several physicochemical and morphological techniques to adequately determine its drug loading Percentage, yield percentage, drug loading efficiency, particle size and zeta potential. Following characters were observed while preparation of nanoparticle suspension. Drug loading %, drug loading efficiency, yield percentage, particle size and zeta potential are shown in table 6 and table 7.

Table 6: Physicochemical characterization of nanoparticles

Formulation Nanoparticle	Drug loading (%)	Loading efficiency(%)	Yield Percentage(%)
FM-1	45.25	65.45	60.32
FM-2	40.20	60.05	65.10
FM-3	45.40	57.25	75.25
FM-4	43.25	55.12	75.12
FM-5	50.20	56.50	60.57

Table 7: Physicochemical characterization of nanoparticles

Formulation	Particle size(nm)	Zeta potential(mv)
FM-1	425	20.25
FM-2	225	20.56
FM-3	356	25.15
FM-4	375	21.65
FM-5	275	25.10

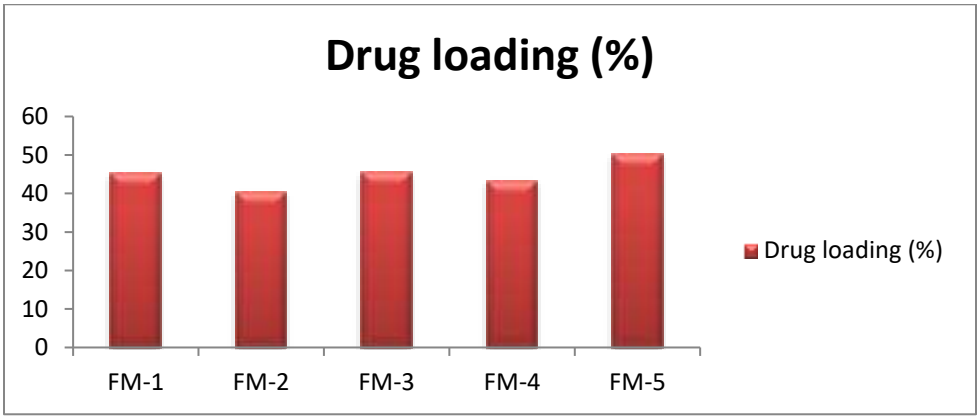

Figure 7: Graph of Drug loading

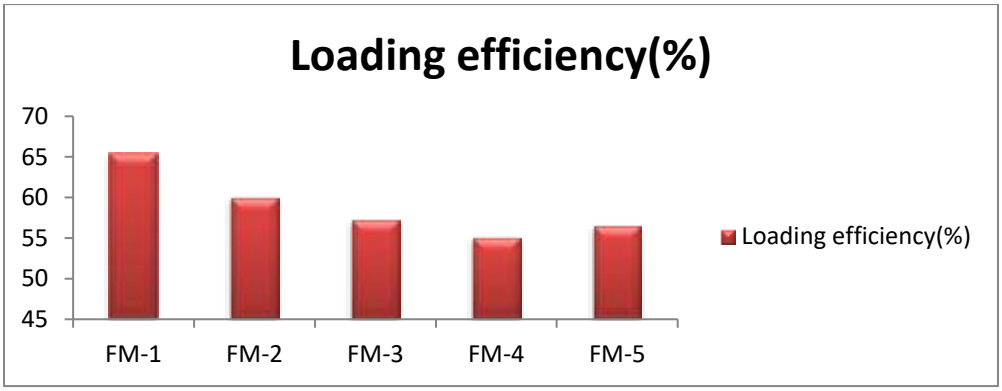

Figure 8: Graph of loading efficiency

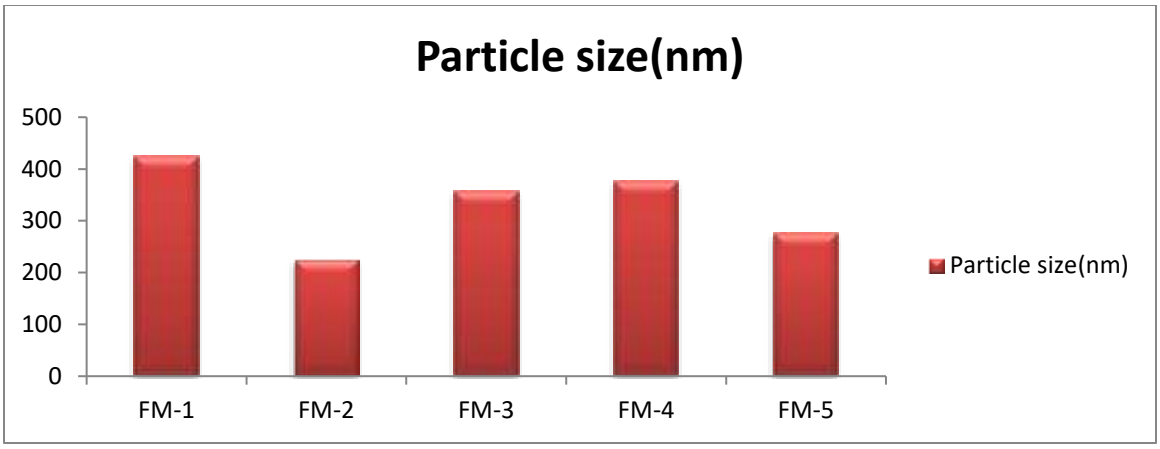

Figure 9: Graph of Particle size

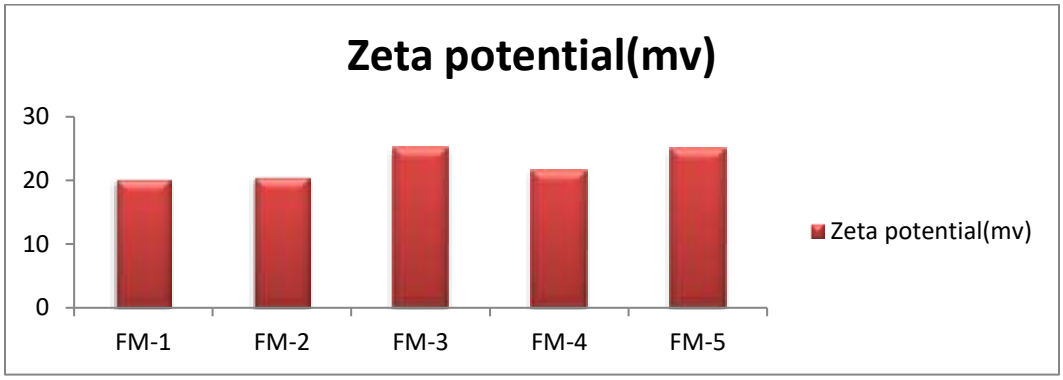

Figure 10: Graph of Zeta potential

Evaluation of Gel Formulation

Gel formulations are used to deliver the drug topically because of easy application, increase contact time and minimum side effects as compare to other topical preparation and oral administration. The evaluation parameters of gel formulation are shown in table 8 and 9.

Table 8: Evaluation of Gel Formulation

Formulation	Ph	Consistency(mm)
NG-1	6.5	5.5
NG-2	5.4	5.1
NG-3	5.3	5.4
NG-4	6.4	6.1
NG-5	5.5	4.5

Table 9: Evaluation of Gel Formulation

Formulation	Spreadability (g.cm./sec.)	Viscosity
NG-1	6.5	8370
NG-2	5.5	8460
NG-3	5.0	8505
NG-4	5.8	8021
NG-5	6.5	7925

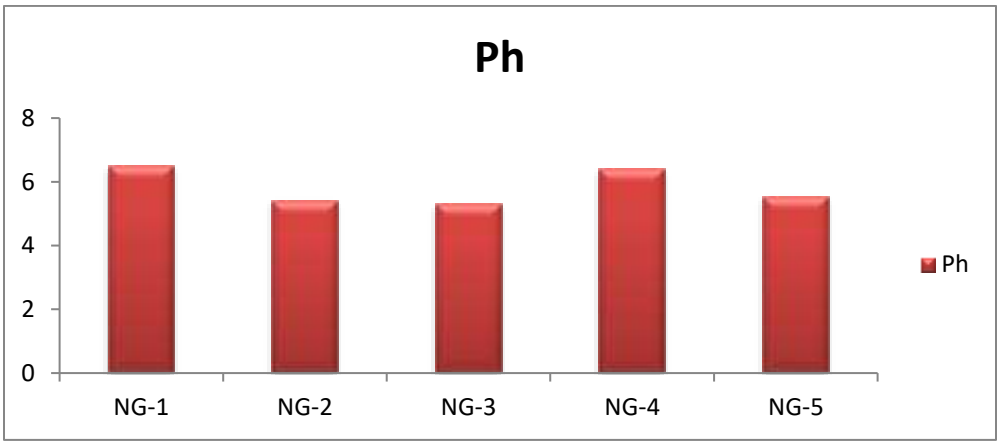

Figure 11: Graph of pH

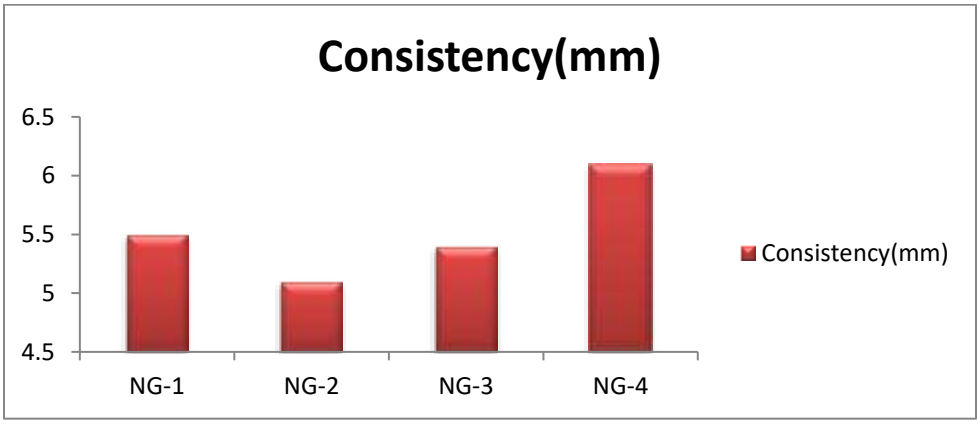

Figure 12: Graph of Consistency

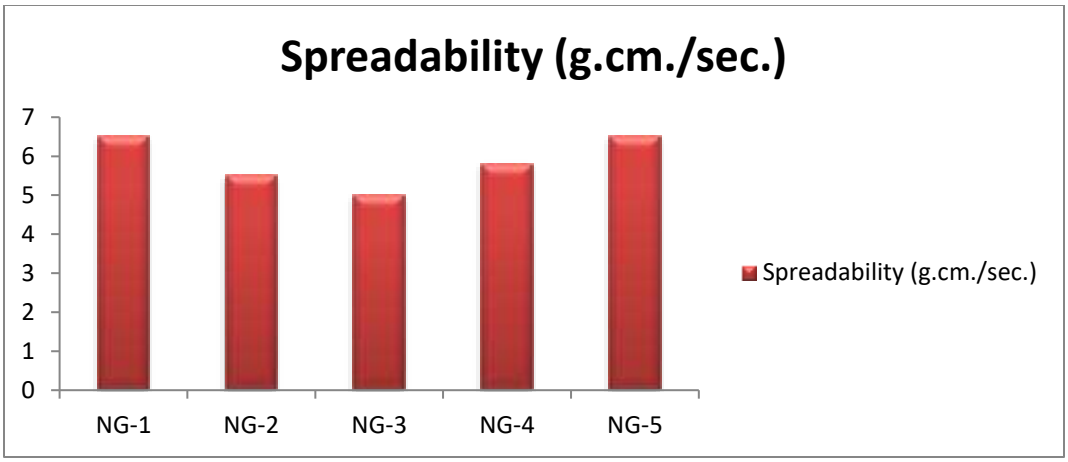

Figure 13: Graph of Spreadability

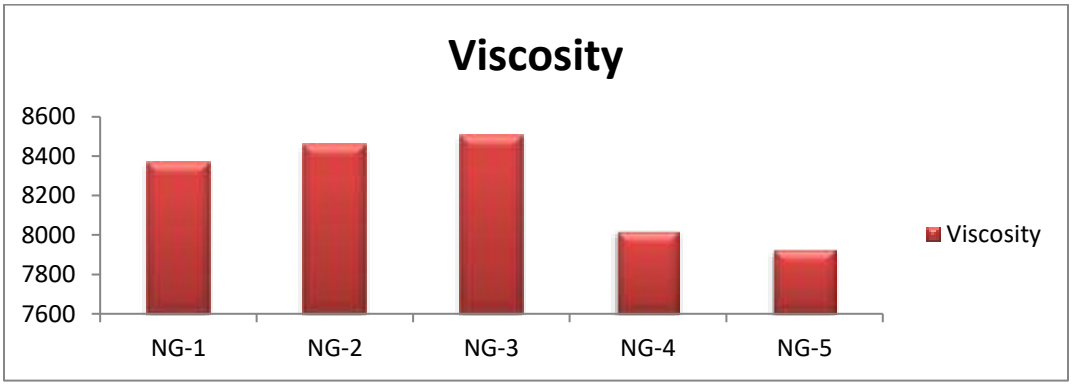

Figure 14: Graph of Viscosity

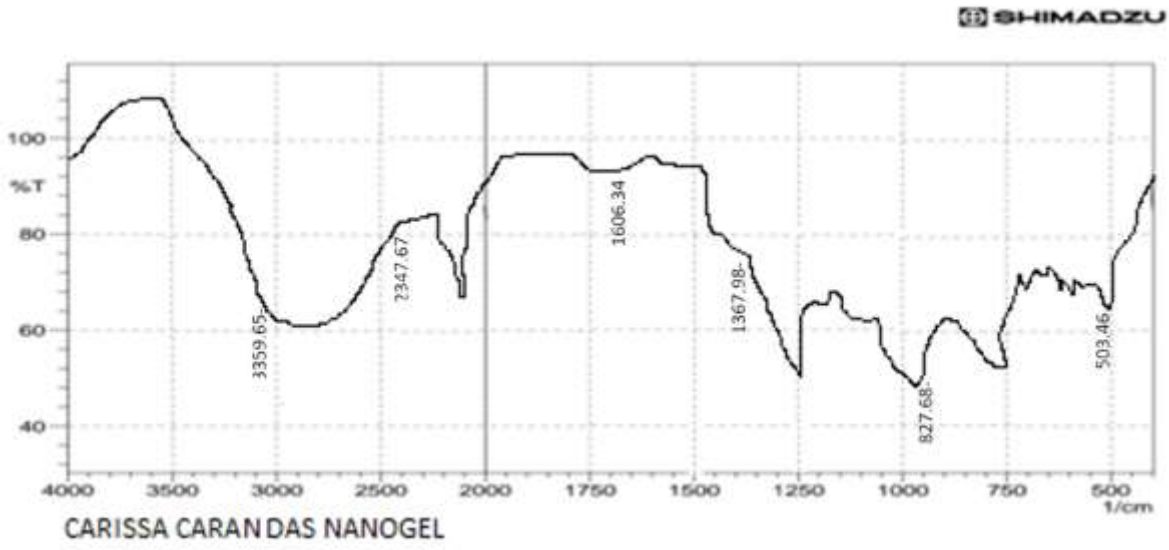

Figure 15: FTIR result for 1:100 ratio of Carissa caranadas Fruit extract

The FT-IR spectrum (Figure 15) of Carissa caranadas show strong IR bands characteristic peaks around 827.68, 1367.98, 1606.34, 2347.67 cm^{-1}, and 3359.65 cm^{-1}. The observed peaks denote the

presence of -C-O-C-, ether linkages, -C-N of aromatic amines, -C=C- group aromatic –C=C- stretch, - NH2 and -OH groups stretch, respectively.

Discussions

Carissa carandas, commonly known as Karonda or Bengal currant, is a medicinal plant that holds significant value in traditional medicine. To ensure the quality and consistency of herbal drugs derived from Carissa carandas, standardization parameters are employed. These parameters provide crucial information about the composition and purity of the plant material.

One important standardization parameter is the ash value, which is determined to be 13.5% w/w for Carissa carandas. Ash value indicates the total inorganic content present in the herbal drug, encompassing both inherent minerals and any extraneous matter. This measurement is essential as it helps determine the purity and authenticity of the plant material.

Another parameter, water-soluble ash, has been found to be 14.56% w/w in Carissa carandas. Water-soluble ash refers to the residue remaining after incinerating the drug and dissolving the resultant ash in water. This parameter provides insights into the water-soluble inorganic constituents present in the plant material. It assists in assessing the quality and determining the presence of any contaminants.

Lastly, the acid insoluble ash of Carissa carandas is measured to be 5.4%. Acid insoluble ash indicates the amount of inorganic matter that remains undissolved even after treating the ash with acid. This parameter helps evaluate the purity and presence of impurities in the herbal drug.

The extractive value using ethanol as the solvent was found to be 10.50%. The extractive value is a measure of the amount of soluble constituents that are extracted from the plant material using a specific solvent.

A preliminary phytochemical screening of Carissa carandas ethanolic extract revealed the presence of alkaloids, tannins, glycosides, and steroids. Flavonoids, phenols, saponins, amino acids and proteins, fixed oils and fats, and carbohydrates were not detected in the extract. These findings provide insights into the potential bioactive compounds present in Carissa carandas.

The physicochemical characterization

The physicochemical characterization of nanoparticles is crucial for evaluating their quality and performance. Several formulations were analyzed, and here are the summarized results:

- Formulation FM-1 exhibited a drug loading of 45.25%, indicating the percentage of drug encapsulated within the nanoparticles. The loading efficiency, which measures the

effectiveness of drug incorporation, was found to be 65.45%. The yield percentage, representing the amount of nanoparticles obtained from the formulation process, was 60.32%.

- In Formulation FM-2, the drug loading was slightly lower at 40.20%, suggesting a lower amount of drug incorporated into the nanoparticles. The loading efficiency was 60.05%, indicating a relatively efficient drug encapsulation process. The yield percentage was slightly higher at 65.10%, indicating a good yield of nanoparticles from the formulation.
- Formulation FM-3 demonstrated a drug loading of 45.40%, indicating a comparable amount of drug incorporation as in FM-1. However, the loading efficiency was slightly lower at 57.25%. Interestingly, the yield percentage was significantly higher at 75.25%, indicating a higher production yield of nanoparticles.
- Formulation FM-4 showed a drug loading of 43.25% and a loading efficiency of 55.12%. The yield percentage was also high at 75.12%, indicating a good yield of nanoparticles.
- Formulation FM-5 had the highest drug loading at 50.20%, suggesting a higher concentration of drug within the nanoparticles. The loading efficiency was 56.50%, indicating a relatively efficient drug incorporation process. The yield percentage was 60.57%, representing a moderate yield of nanoparticles.

The particle size and zeta potential of the nanoparticle formulations were also determined. Here are the results:

- Formulation FM-1 exhibited a particle size of 425 nm, representing the average diameter of the nanoparticles. The zeta potential, a measure of the surface charge of the particles, was found to be 20.25 mV.
- Formulation FM-2 had a smaller particle size of 225 nm, indicating a smaller average diameter of the nanoparticles. The zeta potential remained relatively constant at 20.56 mV.
- Formulation FM-3 demonstrated a particle size of 356 nm, representing the average diameter of the nanoparticles. The zeta potential increased slightly to 25.15 mV compared to the previous formulations.
- Formulation FM-4 showed a particle size of 375 nm, similar to FM-3. The zeta potential remained stable at 21.65 mV.

- Formulation FM-5 exhibited a particle size of 275 nm, indicating a smaller average diameter of the nanoparticles compared to FM-1 and FM-3. The zeta potential increased to 25.10 mV.

The evaluation of gel formulations involved assessing various parameters related to pH, consistency, spreadability, and viscosity. Here are the results obtained:

The pH of the gel formulations varied between 5.3 and 6.5, indicating slightly acidic to neutral conditions. Consistency, measured in terms of millimeters, ranged from 4.5 to 6.1, reflecting the firmness or solidity of the gels.

Spreadability, expressed in grams per centimeter per second (g.cm./sec.), varied between 5.0 and 6.5. Spreadability assesses the ease of spreading the gel formulation, with higher values indicating better spreadability.

Viscosity, measured in centipoise (cps) or millipascal-seconds (mPa•s), ranged from 7925 to 8505. Viscosity determines the flow characteristics of the gel and is an important parameter for formulation stability and ease of application.

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